AMERICAN ZOOLOGIST

Volume 1, Number 2 May 1961

# AMERICAN SOCIETY OF ZOOLOGISTS OFFICERS—1961

## GENERAL OFFICERS

| | | |
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| President: | C. Ladd Prosser<br>University of Illinois<br>Urbana, Illinois | 1961 |
| President-Elect: | Curt Stern<br>University of California<br>Berkeley, California | 1961 |
| Past-President: | Emil Witschi<br>State University of Iowa<br>Iowa City, Iowa | 1961 |
| Secretary: | Charles B. Metz<br>Florida State University<br>Tallahassee, Florida | 1963 |
| Treasurer: | Jerry J. Kollros<br>State University of Iowa<br>Iowa City, Iowa | 1962 |
| Program Officer: | Ray L. Watterson<br>Northwestern University<br>Evanston, Illinois | 1962 |

## DIVISIONAL OFFICERS

### *Animal Behavior*

| | | |
|---|---|---|
| Chairman: | William C. Young<br>University of Kansas<br>Lawrence, Kansas | 1961 |
| Vice-Chairman: | William C. Dilger<br>Cornell University<br>Ithaca, New York | 1961 |
| Secretary: | Martin W. Schein<br>Pennsylvania State University<br>University Park, Pennsylvania | 1962 |

### *Comparative Endocrinology*

| | | |
|---|---|---|
| Chairman: | Aubrey Gorbman<br>Barnard College<br>New York, New York | 1961 |
| Chairman-Elect: | Berta V. Scharrer<br>Albert Einstein College of Med,<br>New York, New York | 1962 |
| Program Officer: | Paul A. Wright<br>University of New Hampshire<br>Durham, New Hampshire | 1962 |
| Secretary: | Howard A. Bern<br>University of California<br>Berkeley, California | 1963 |

### *Comparative Physiology*

| | | |
|---|---|---|
| Chairman: | Theodore H. Bullock<br>University of California<br>Los Angeles, California | 1961 |
| Chairman-Elect: | Arthur D. Hasler<br>University of Wisconsin<br>Madison, Wisconsin | 1961 |
| Program Officer: | Kenneth D. Roeder<br>Tufts College<br>Medford, Massachusetts | 1962 |
| Secr.-Treas.: | Frank J. Vernberg<br>Duke University<br>Durham, North Carolina | 1961 |

### *Developmental Biology*

| | | |
|---|---|---|
| Chairman: | James D. Ebert<br>Carnegie Institution<br>Baltimore, Maryland | 1961 |
| Vice-Chairman: | John A. Moore<br>Columbia University<br>New York, New York | 1961 |
| Program Officer: | John W. Saunders<br>Marquette University<br>Milwaukee, Wisconsin | 1962 |
| Secr.-Treas.: | Beatrice Mintz<br>Institute for Cancer Research<br>Philadelphia, Pennsylvania | 1962 |

### *Invertebrate Zoology*

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|---|---|
| Chairman: | Clarence J. Goodnight<br>Purdue University<br>Lafayette, Indiana |
| Vice-Chairman: | Bertil G. Anderson<br>Pennsylvania State University<br>University Park, Pennsylvania |
| Program Officer: | Melbourne R. Carriker<br>University of North Carolina<br>Chapel Hill, North Carolina |
| Secr.-Treas.: | Charles F. Lytle<br>Tulane University<br>New Orleans, Louisiana |

### *Vertebrate Morphology*

| | | |
|---|---|---|
| Chairman: | D. Dwight Davis<br>Chicago Nat. Hist. Museum<br>Chicago, Illinois | 1961 |
| Vice-Chairman: | Harvey Fisher<br>Southern Illinois University<br>Carbondale, Illinois | 1961 |
| Secr.-Treas.: | Howard Evans<br>Cornell University<br>Ithaca, New York | 1962 |

## EXECUTIVE COMMITTEE

The six general officers of the Society; the Chairmen of the Divisions (ex officio); and the following members-at-large (one replacement, only, to be elected in 1961 and 1962 to stabilize to three members-at-large in 1963).

| | |
|---|---|
| Lewis H. Kleinholz | 1961 |
| Edgar Zwilling | 1961 |
| Edgar J. Boell | 1962 |
| Kenneth W. Cooper | 1962 |
| Graham P. DuShane | 1963 |

## PUBLICATIONS COMMITTEE

This committee also functions as the Editorial Board of *American Zoologist*.

| | |
|---|---|
| Sheldon J. Segal (Chairman) | 1961 |
| David W. Bishop | 1962 |
| John A. Moore | 1963 |
| Elmer G. Butler | 1964 |
| Daniel Mazia | 1965 |

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|---|---|
| Sears Crowell (ex officio) (Man'g Ed., *American Zoologist*) | 1965 |
| Arthur W. Pollister (ex officio) (Man'g Ed., *J. Morphol.*) | 1964 |

JOURNAL OF MORPHOLOGY

| | |
|---|---|
| Arthur W. Pollister, Managing Editor | 1964 |
| G. H. Ball | 1961 |
| J. H. Lockhead | 1961 |
| M. Singer | 1961 |
| R. A. Miller | 1962 |
| A. Novikoff | 1962 |
| H. M. Smith | 1962 |
| H. V. Crouse | 1963 |
| C. F. Lytle | 1963 |
| P. L. Risley | 1963 |

POLICY COMMITTEE

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|---|---|
| C. L. Prosser (President) | 1961 |
| C. Stern (President-Elect) | 1962 |
| Edgar J. Boell | 1961 |
| William Trager | 1961 |
| Arthur W. Martin | 1961 |

EDUCATION COMMITTEE

| | |
|---|---|
| Allyn Watterman (Chairman) | 1961 |
| Roland Walker | 1961 |
| Arthur Martin | 1962 |
| J. R. Harrison | 1962 |
| K. A. Stiles | 1963 |
| W. Balamuth | 1963 |
| N. L. Bennington | 1963 |

MEMBERSHIP COMMITTEE

| | |
|---|---|
| John W. Saunders (Chairman) | 1962 |
| Martin W. Schein | 1961 |
| John Anderson | 1963 |
| Charles B. Metz (ex officio) | 1963 |

LOCAL CHAIRMAN

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|---|---|
| AIBS, Purdue, M. X. Zarrow | 1961 |

REFRESHER COURSE

| | |
|---|---|
| D. Kennedy | 1961 |

REPRESENTATIVES TO OTHER ORGANIZATIONS

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| Division of Biology and Agriculture of the National Research Council: Theodore L. Jahn | 1962 |
| Council of American Association for the Advancement of Science: David W. Bishop | 1961 |
| Albert Wolfson | 1962 |
| Board of Governors of the American Institute of Biological Sciences: W. Gardner Lynn | 1963 |
| Biological Stain Commission: Harold W. Beams | 1962 |
| Board of Trustees of the American Type Culture Collection: A. M. Elliott | 1963 |

## EDITOR'S NOTE

This, the second issue of *American Zoologist*, contains papers from two symposia of the Society held in New York in December, 1960.

The symposium on "Evolution and dynamics of vertebrate feeding mechanisms" was organized by Perry W. Gilbert and Bobb Schaeffer for the Division of Vertebrate Morphology.

The symposium on "Physiology of molluscs" was organized by R. Boolootian for the Division of Comparative Physiology.

The editor appreciates the cooperation of organizers and authors in the preparation of the papers for this issue.

## PROCEEDINGS OF THE FIFTY-SEVENTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF ZOOLOGISTS

The 57th Annual Business Meeting of the Society was called to order by the President, Professor Emil Witschi, in the Commodore Hotel, New York, N. Y., at 4:30 pm immediately following the Business Meeting of Section F of the AAAS. The President announced the following results of the recent election: President-Elect, Dr. Curt Stern; Secretary, Dr. Charles B. Metz; Member-at-large of the Executive Committee, Dr. Graham P. DuShane. The President also announced various appointed representatives of the Society on the boards of other organizations, membership on various society committees, and editors and associate editors of society journals. These appointees will be found named elsewhere in this issue.

Dr. Edgar J. Boell, Chairman of the Committee to Revise the Constitution, explained the major changes that had been made, and answered questions. The Revised Constitution, which had been previously mailed to the membership, was passed unanimously, well over one hundred members being present at the time of the vote. As has been true since prior to World War I, when the Society was very much smaller, 25 members constituted a quorum. The Revised Constitution doubles the number required.

Dr. Sears Crowell, Managing Editor of the Society's new quarterly, *American Zoologist*, made several announcements. The first issue will appear sometime this spring

and carry papers from the symposium on Metamorphosis. This issue is expected to run to about 155 pages in two-column format. The final vote for establishing the quarterly was 1,176 *for,* 95 *against,* and about 730 *not heard from.*

It was announced that plans were well advanced to continue, on an expanded scale, regional conferences in developmental biology. The Division of Comparative Endocrinology is actively seeking funds for regional conferences. A Division of Invertebrate Zoology is in the process of formation.

The President announced that Dr. John A. Moore of Columbia University has accepted the post of Chairman of the Program Committee for the XVIth International Congress of Zoology, which will be held in 1963. Dr. Gerard Piel, publisher of the *Scientific American,* has become Chairman of the Finance Committee for the Congress.

Resolutions were passed congratulating Dr. H. J. Muller on the occasion of his 70th birthday, thanking Dr. Harry Charipper for his services as Local Representative, and thanking Dr. Dael Wolfe for the part the AAAS played in making our meetings a success.

It was moved, seconded, and passed after brief discussion, that the Society go on record as opposed to the so-called Cooper Bill, which would hamper the use of animals in research and teaching, and that copies of the resolution be sent to the proper governmental officers. The resolution follows:

Senate Bill 3570 would regulate the use of all vertebrate animals in research and teaching. Such legislation would reduce freedom in day-to-day planning and conduct of research and would reduce the amount of research in many areas which provide basic information for medicine, agriculture, and the conservation of animal resources. This proposed legislation is discriminatory in its implication that animal biologists are suspect and inhumane in laboratory practice. The American Society of Zoologists, assembled in convention in New York City in December, 1960, strongly opposes Senate Bill 3570.

It was moved, seconded, and passed that the Society urge governmental support of research and free access to medical information, and the training of medical personnel in the field of birth control. After further discussion it was agreed almost, but not quite, unanimously to accept the motion, which had already been passed by a clear majority, and to send it to a committee, to be appointed by the President, to be reworded before publication. This was done. The resolution in its definitive form follows:

The American Society of Zoologists views the mounting rate of population growth, especially in the world's poorest areas, as a principal factor contributing to global conditions of human misery, famine, and under-education, and we urge our government to adopt policies in keeping with this country's tradition of deep sympathy for human suffering.

In many countries, officially adopted policies of voluntary fertility control are rendered ineffectual, and virtually in all countries, including our own, the desire of some segments of the populace for smaller families remains unfulfilled because of the lack of simple, acceptable methods for the voluntary regulation of human reproduction. The freedom to limit family size is still the privilege of the educated few and should be extended to all people. Furthermore, the distribution of scientific and medical information should be unencumbered by restrictive laws or prejudicial attitudes.

As biological scientists, we recommend that our Government implement a policy indicative of its dedication to the service of mankind by assuring the full support of its appropriate agencies to research in the biology of reproduction and fertility control, and by offering programs of assistance in these fields for the training of American as well as foreign scientists.

The Treasurer, Dr. Jerry J. Kollros, presented the annual financial report and a proposed budget for 1961 which had been approved earlier by the Executive Committee.

President Witschi announced that the books of the Society had been examined by Dr. Titus C. Evans and Dr. Richard V. Bovbjerg and found to be in good order. The Treasurer's report and the budget were then accepted.

The Annual Business Meeting then adjourned to permit the members to attend the joint AAAS Section F and ASZ Dinner. Dr. Viktor Hamburger, Vice President for Section F, presented an informative and delightful address entitled "An Embryologist Visits Japan."

At its meeting after the dinner and in accordance with the provisions of the Revised Constitution, the Executive Committee elected the following outstanding zoologists Corresponding Members: Dr. B. L. Astaurov of Moscow, Dr. F. Baltzer of Bern, Dr. Louis Gallien of Paris, Dr. Giuseppe Montalenti of Napoli, Dr. F. C. A. Pantin of Cambridge, Dr. B. Rensch of Munster, Dr. Paulo Sawaya of Sao Paulo, Dr. Gunnar Thorson of Copenhagen, Dr. N. Tinbergen of Oxford, and Dr. Tohru Uchida of Sapporo.

Respectfully submitted,
GAIRDNER B. MOMENT
*Secretary*

*Treasurer's report*
August 16, 1959 through December 15, 1960

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| **CREDITS** | |
| Cash balance August 16, 1959 | $ 5,926.35 |
| Receipts from dues | 12,516.00 |
| Sale of abstracts | 18.00 |
| Sale of careers brochures | 766.47 |
| U. S. Series F bond M164 8698F (purchase price) | 740.00 |
| Grants: | |
| NSF #10085—printing & distributing brochures | 2,456.00 |
| NSF #10811—Regional Conf. in Develop. Biology | 4,000.00 |
| *NSF #12558—Refresher course, "Metamorphosis" | 4,900.00 |
| †NIH RG-7814—Symposium, "Spermatozoan Motility" | 9,020.00 |
| Interest on bank balances | 120.25 |
| Refunds, premiums, miscellaneous receipts | 74.38 |
| | $40,537.45 |
| **DEBITS** | |
| Grants: | |
| NSF #7898 (refund to NSF) | $ 91.11 |
| NSF #7600 (including refund to NSF of $248.33) | 750.00 |
| NSF #10085 | 2,456.00 |
| NSF #10811 (including refund of $122.92) | 4,000.00 |
| NSF #12558 | 2,807.22 |
| NIH RG-7814 | 2,385.00 |
| Wistar Institutes—2 sets of Abstracts | 3,543.54 |
| AIBS dues | 1,875.00 |
| Expenses, Secretary | 1,374.48 |
| Expenses, Treasurer (other than for grants) | 405.24 |
| Expenses, Program Officer | 760.19 |
| Expenses, other officers | 143.94 |
| Expenses, local representative (N. Y. meeting) | 100.00 |
| Expenses, Editor | 300.00 |
| Divisional expenses: | |
| Developmental Biology (2 years) | 100.00 |
| Comparative Endocrinology | 64.62 |
| Behavior | 49.58 |
| Comparative Physiology | 83.77 |
| Newsletters | 50.37 |
| Refunds of excess dues payments | 17.50 |
| U. S. Savings Bond M164 8698F | 740.00 |
| | $22,097.56 |
| ‡Bank balance,* December 15, 1960 | $18,439.89 |

* Includes $637 for indirect expenses ("overhead")
† Includes $820 for indirect expenses
‡ Includes $54.00 to be paid towards expenses of the next International Congress of Zoology, and the unexpended balances of NSF Grant #12558 and of NIH Grant RG-7814

Am. Zoologist. 1:177-181 (1961).

# FEEDING MECHANISMS OF AGNATHA AND EARLY GNATHOSTOMES

Robert H. Denison
*Chicago Natural History Museum*

Before embarking on a discussion of the feeding mechanisms of Agnatha, it is of interest to speculate on the manner of feeding of early vertebrate ancestors. Jawless vertebrates, both living and fossil, feed or fed in a number of different ways, most of which are considered to be specialized. On the other hand, the protochordates are quite uniform and probably primitive in their method of obtaining food. Most of them feed on small particles suspended in sea water. The water is drawn into the mouth and pharynx by means of a cilia-induced current, and food particles are trapped and carried to the alimentary tract by strings or sheets of mucus. The water that has passed through the mucus trap escapes through the gill slits, which are not primarily respiratory, but a part of the food straining device. In both tunicates and *Branchiostoma* this type of feeding is typically developed.

Microphagous feeding employing ciliary currents and mucus entanglement is well adapted to small aquatic forms leading a sedentary life. It is common in many groups of sessile marine invertebrates, but with one exception there is nothing of this sort among the vertebrates. The exception is the ammocoete larva of the lampreys (Newth, 1930). Ammocoetes live in burrows and feed on micro-organisms and other food particles suspended in the water. A water current is drawn into the mouth, passed through the pharynx, and out through the gills; however, in contrast to the protochordates, the current is not produced by cilia but by muscles of the pharynx and velum. Food is trapped from the water current by a mucus net, much as in *Branchiostoma*. The mucus strands are carried along by ciliary tracts, which form them into a cord that passes down the pharynx into the gullet. The occurrence of this type of microphagous feeding in ammocoetes, in protochordates, and in many invertebrate groups supports the belief that it was employed by some of the ancestors of vertebrates. The latter were probably rather inactive, bottom-living types.

The adults of living Agnatha, or cyclostomes, are highly specialized in their manner of feeding. This is particularly true of the lampreys, which are parasites, attaching by suction to a fish and feeding on its blood and tissue. The attachment is by the oral disc, and the suction is produced by closing the velar valve at the mouth of the pharyngeal tube, and then by forcing water out of the oral cavity, using the hydrosinus as a pump (Reynolds, 1931). While the lamprey is attached to its prey, the breathing and feeding mechanisms are entirely separated by the velar valve. In the oral cavity pressure is low so that suction will maintain the attachment. Here the powerful tongue with its horny teeth rasps the skin and scales of the prey, while buccal glands produce an anticoagulating secretion. In the pharyngeal tube behind the velar valve pressure is normal, and breathing is accomplished by rhythmical elastic expansions and muscular contractions of the gill basket. Water is drawn in through the external tubes into the sac-like gills and is expelled through the same tubes.

Hagfishes are scavengers rather than parasites. Since they have no means for attaching themselves to prey, they are largely restricted to eating dead or disabled fishes, though some feed at times on marine worms (Gustafson, 1935). They feed by means of a pair of toothed plates, which are opened and closed like the leaves of a book, tearing off pieces of flesh. In this way they rasp their way into a fish, usually starting at the abdominal cavity. When not feeding, the velar folds pump a continuous respiratory current of water into the terminal nostril, through the pharynx and gill sacs, and out through the branchial tubes and apertures.

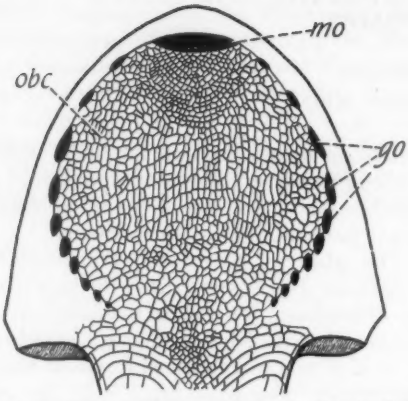


FIG. 1. Ventral side of shield of *Hemicyclaspis murchisoni* (× 1); modified slightly after Stensiö, 1932. go, gill openings; mo, mouth; obc, small plates of flexible ventral cover of oralo-branchial region.

When they are feeding, their nostril is usually pressed against or buried in a fish and thus cannot serve as an intake for the respiratory current. According to Strahan (1958), they back out of the fish about every three or four minutes, presumably to breathe. Cutaneous respiration appears to be adequate while they are imbedded in their meal.

Three major groups of fossil Agnatha are known in the Silurian and Devonian, the Osteostraci, Anaspida, and Heterostraci. Best known morphologically are the Osteostraci, typified by *Cephalaspis* and *Hemicyclaspis,* which are similar adaptively to the living South American catfishes, *Loricaria* and *Plecostomus.* The general adaptation of *Hemicyclaspis* (Fig. 1)—its flat bottom, ventral mouth, dorsal nostril, and dorsal eyes—indicates that it lived and fed on the bottom. It probably was not a selective feeder, since apparently it had no mouth mechanism by which it could have picked up separate pieces of food. Its feeding could have been suctorial, the mouth functioning simply to open and close. The suction may have been provided by the flexible floor of the oralo-branchial chamber (Fig. 1, *obc*), worked as a pump by internal muscles attached to the interbranchial ridges (Watson, 1954), or to the tops of the extrabranchial atria (Stensiö, 1958). If this interpretation is correct, *Hemicyclaspis* probably sucked in large quantities of mud (Heintz, 1939), as does the living *Plecostomus.* Branchial filters of some sort, presumably gill rakers, would have been necessary to filter out the mud as the water passed through the gills.

There have been other theories about the feeding habits of Osteostraci. Gregory (1946) suggested that they fed by ciliary currents, but it is probable that Osteostraci were too large and active for this method to have supplied adequate nourishment. Kiaer (1928) favored a biting or crushing mouth, but the known mechanism is inadequate for this type of feeding. Gregory (1951) thought that they may have been parasitic and attached to their prey by mouth suction; this is unlikely as it would have required many unknown adaptations —for creating the suction, for breathing while feeding, for rasping the prey, and others. Stensiö (1958) believed that Osteostraci had mobile tongues, rasping in one group. He based this belief on the inference that there must have been a ventral tooth plate to oppose the so-called "maxillary tooth plate," but there is no evidence for this.

A second group of fossil Agnatha, the Anaspida, are more fish-like forms related to Osteostraci. Their internal anatomy is unknown and there have been many different interpretations of their morphology. Kiaer (1924) restored the mouth as a terminal, slit-like structure, surrounded by dermal plates externally, and with internal cartilaginous supports. He believed that they were able to bite relatively large prey. Though not accepting Kiaer's implication of gill arch support for the jaws, Gregory (1951) and Watson (1954) have considered a biting or nibbling function of the mouth as probable. Moy-Thomas (1939) suggested that anaspids may have been plankton feeders, but their small mouth and gills make this unlikely. In recent years Heintz (1958), Parrington (1958), and Stensiö (1958) have independently restored the anaspid mouth as a terminal vertical slit or

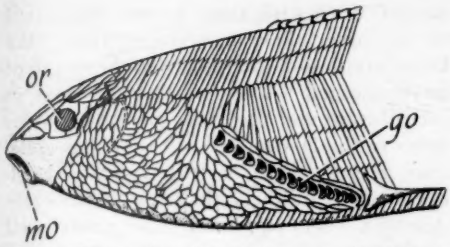


FIG. 2. Restoration of anterior part of body of *Pharyngolepis oblongus* (× 1); modified slightly after I. C. Smith, 1957. go, gill openings; mo, mouth; or, orbit.

ovoid opening in soft tissues (Fig. 2, *mo*). Heintz believed that the manner of feeding was similar to that of *Branchiostoma* and ammocoetes—that is, that they were microphagous, employing ciliary currents and mucus entanglement. This seems unlikely since anaspids were surely very active forms for which this type of feeding would be inadequate. Stensiö has produced an elaborate restoration, based on lampreys, providing the anaspids with a rasping tongue and a suctorial mouth; this is largely hypothetical since there is little direct evidence for such structures. Bystrow (1956) and Parrington (1958) believed that the mouth was adapted for sucking up food from the bottom, and the latter provided them with a suction apparatus in the "chin development." Bottom feeding with a terminal mouth would require a position with the tail elevated, but the strongly developed hypocercal tail makes it improbable that such a position was typical in this group. The action of the tail in swimming would result in driving the tail end down, and its function may have been in part to provide the angle of attack needed for lift in swimming. It is certainly not adapted for maintaining the tail-up position shown in Bystrow's and Parrington's restorations.

If the anaspids did not have mechanisms for biting or nibbling, if they were too large and active to be adequately nourished by ciliary currents and mucus entanglement of small particles, and if they were not adapted for plankton or bottom feeding, about the only possibility left is suctorial feeding of some sort. It is not impossible that their feeding was similar to that of some living cyclostomes, though it is unlikely that they were as highly specialized for parasitism as are lampreys. They are certainly related and possibly ancestral to lampreys. At least one anaspid, *Pharyngolepis*, tended towards an eel-like shape. Their gills, as far as they can be reconstructed (Fig. 2, *go*), must have had many similarities to those of lampreys. And there is space in front of the gills for the development of some special structure, possibly a suction device, or even the rasping tongue in which Stensiö believes.

A third group of fossil Agnatha, the Heterostraci, also lack any preservable endoskeleton, so little is known about their internal structure. In the families Pteraspidae and Cyathaspidae a number of oral plates (Fig. 3B, *op*) are arranged in a row along the posterior border of the ventrally placed mouth. These plates are covered with dentine ridges not only on their external or ventral faces, but also on all but the posterior part of their dorsal faces. This means that they were free to move with re-

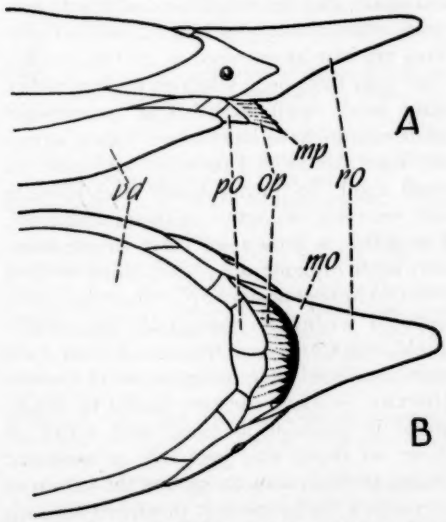


FIG. 3. Anterior part of *Pteraspis rostrata* (× 5/4); after White, 1935. A, lateral view; B, ventral view. mo, mouth; mp, mouth protruded; op, oral plates; po, postoral plates; ro, rostral plate; vd, ventral disc.

spect to each other, and that they were attached by muscles only in their postero-dorsal parts, though they probably were completely united by skin. Opposite the antero-internal ends of the oral plates, the internal surface of the rostrum is covered with denticles. This area was called the "maxillary tooth plate" by Kiaer (1928), who believed that the oral plates worked against it, and that these forms had a biting or crushing mouth. Stensiö also believed that pteraspids had a biting mouth, but his restoration (1958, Fig. 190), based on myxinoids, shows the oral plates working against a hypothetical palato-subnasal lamina which surely did not exist.

Others have questioned the ability of the oral plates to bite, and I agree that they could at best form rather feeble grasping or holding organs. Moreover, there is no evidence of wear of the denticles on either the oral or rostral plates. White (1935, pp. 49-50) has suggested that the mouth was protrusible and that the oral plates could be moved anteroventrally and splay out to form a scoop (Fig. 3A, *mp*) for feeding in the bottom mud. It is probable that most pteraspids and cyathaspids were largely bottom feeders, though their benthonic adaptations are not as extreme as in Osteostraci. They may have been selective feeders rather than mud swallowers. The protrusible mouth could have been more than a scoop, and have functioned to select and pick up small food, including small invertebrates that were not too active in swimming away. The gills, as shown by their impressions, were high, of moderate width, but short and crowded antero-posteriorly. Since they were enclosed within an essentially immovable shield, respiratory currents could not have been produced by movements of the whole pharynx. Ciliary currents would be inadequate in animals as large and active as these, so there was probably a muscular pump, perhaps something like the velum of myxinoids. The current produced by such a pump may have had an important function in picking up food from the bottom.

Living and fossil Agnatha illustrate a variety of feeding adaptations, but the possibilities were limited, particularly for predation, until such time as jaws supported by an inner skeleton were evolved. We know nothing of the history of the origin of gnathostomes. Acanthodians appeared in the Silurian and the other groups of jawed fishes in the Devonian, with their jaw skeletons already more or less typically developed. It is unlikely that any of the early Agnatha that we know reasonably well could have been ancestral to gnathostomes.

For one of the Agnatha, the first step towards gill-arch jaws must have been an enlargement of the mouth, at first an adaptation only for taking larger food and not necessarily for predation. As the mouth enlarged, it would crowd backwards into the first, and then the second gill arch. The first arch, as Stensiö has shown in Osteostraci, was innervated by the profundus nerve, and is premandibular, while the second, innervated by the trigeminus, is the mandibular arch of all gnathostomes. Thus the second arch would come to surround the mouth as the palato-quadrate and Meckel's cartilage (Fig. 4), and would act as a place of attachment for jaw muscles and a base for teeth as they evolved from dermal denticles. A mechanical difficulty of this arrangement is that the hinge between the upper and lower jaws is not firmly supported. This was remedied when the enlarging mouth crowded back to the third or hyoid arch, part of which evolved into a brace, the hyomandibula, between the skull and the jaw articulation. Watson (1937) believed that Acanthodii and Placodermi were in the evolutionary stage before the hyoid arch was modified to aid in the support of the jaws. He called them Aphetohyoidea, and claimed that their hyoid arch was still a typical gill arch with a widely open gill slit between it and the mandibular arch. In another connection I have considered Watson's contention in detail, and came to the following conclusions: 1) In the Acanthodii the prehyoidean gill was reduced, the main operculum was probably carried on the hyoid arch as in Osteichthyes, and the hyoid arch may have aided in jaw suspension. Thus I do not believe that they were aphetohyoids. 2) It is possible that some Arthrodira were aphetohy-

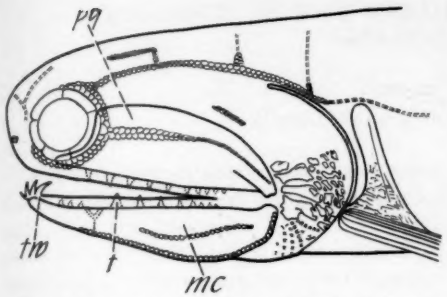


FIG. 4. Head of *Ischnacanthus gracilis* (× 5/2), a Lower Devonian acanthodian; after Watson, 1937. mc, Meckel's cartilage; pq, palatoquadrate; t, jaw tooth; tw, symphyseal tooth whorl.

oidean, though this cannot be demonstrated now. Their gill cover was borne by the mandibular arch. The jaw articulation was supported largely by the dermal bones of the cheek, and the hyoid arch may not have been involved, except perhaps in the specialized Rhenanida.

The Acanthodii show a relatively limited range of feeding adaptation. Probably many of them were predaceous, feeding on other fishes or on invertebrates. Their mouths were large or moderately large and their teeth were large and pointed in some (Fig. 4), small or absent in others. A specimen of a large acanthodian in the British Museum has inside it the remains of a small *Cephalaspis* that apparently had been eaten.

Placodermi show a wide adaptive radiation. Some arthrodires, such as *Arctolepis*, were small-mouthed bottom dwellers, similar adaptively to Osteostraci, and may have been mud swallowers. Some, as *Dunkleosteus*, became huge predators with slicing jaws; others (Ptyctodontida, *Mylostoma*) had a crushing dentition and may have fed on shelled invertebrates. The ray-like Rhenanida were probably bottom feeders. The Antiarcha lived on stream bottoms and fed on mud; the swallowed mud has been found in *Bothriolepis* and shows that they had a spiral valve in their intestine.

This brief review has covered three major stages in the evolution of feeding mechanisms of early vertebrates:

1) An ancestral stage in which relatively small and inactive forms may have fed on minute particles in sea water by means of a cilia-induced current and mucus entanglement. Most protochordates feed in this manner, but the only living vertebrate retaining any trace of it is the ammocoete larva of the lamprey.

2) A jawless stage in which ostracoderms and cyclostomes show a limited range of adaptation to bottom feeding, mud swallowing, scavenging, and parasitism.

3) And finally the gnathostome stage in which gill-arch jaws permitted a much wider variety of feeding adaptation, particularly to predation.

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# DYNAMICS OF THE FEEDING MECHANISM OF LARGE GALEOID SHARKS

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A few species of sharks have jaws structurally effective for crushing hard-shelled prey, but the functions of the jaws and teeth of the remaining species, that is of most kinds of sharks, are restricted to grasping prey or to cutting off pieces of prey to get something of suitable size for swallowing. The grasping function is one which may be performed by any shark; but the type of jaw and dentition apparently most effective and most restricted for this purpose is that found in the sand shark, *Carcharias taurus*, one of the more primitve of existing galeoids.

The sand shark has teeth especially adapted for holding. The teeth are long, spike-like, and irregularly spaced and are held permanently in position to function in more than one series. The sand shark has heavily calcified, dense jaws which may be protruded, but the upper jaw has no strong cartilaginous processes. Thus it has little support to resist lateral strain, since it lacks these strong processes which characterize the more advanced hyostylic jaw suspension of most common galeoids.

The sand shark feeds on fishes and invertebrates small enough to be swallowed entire. Although it is accused of attacking larger prey, little definite evidence of this exists and it is probable that such occurrences, if they happen at all, are accidental. The sand shark is the only large shark which, at least in the Florida area, is regularly successful in feeding on littoral gamefish such as the relatively fast-swimming and elusive seatrouts, bluefish, Spanish mackerel, and similar species. Possibly the effectiveness of the jaws accounts in part for the ability of the species to feed on fishes which normally elude all other sharks. Sand sharks are said, however, to work together in groups to surround schools of fishes (Radcliffe, 1916) and this idea of cooperation is supported by accounts of sand shark feeding given me by Florida fishermen. If cooperative effort in feeding is not involved, it is difficult to imagine how the relatively stiff and cumbersome sand shark could catch such fishes.

When attacking large prey, vertebrate predators having dentition that is effective primarily for grasping often merely bite down on a portion of the prey and twist off that portion by rotating their entire bodies. Observations of hooked sand sharks and netted sand sharks indicate that the species is not a twister—that is, it does not grasp objects and roll or spin. Sand sharks apparently are not structurally suited to spinning. Other large galeoid sharks such as the lemon shark, *Negaprion brevirostris*, and the mako, *Isurus oxyrinchus* (both species having spike-shaped teeth somewhat similar to those of the sand shark but having processes of the upper jaw which strengthen it against lateral strain) do feed to a large extent on relatively small fishes. But these sharks also regularly attack larger prey. The lemon shark shakes or twists off pieces while the mako, a species not suited by its fin arrangements or body form for twisting, merely vibrates its head region.

Cutting teeth of sharks are usually arranged in saw-like bands. The bite of large sharks is probably not powerful enough to cut off pieces of tough prey such as a porpoise, sea-turtle, or whale merely by closing the jaws. Cutting tough prey or cutting through bony structures requires heavy saw-like strokes. A few kinds of sharks have cutting bands in both upper and lower jaws. The tiger shark, *Galeocerdo cuvieri*, seems to accomplish the sawing effect by twisting its body in a kind of spiral which produces a sawing action by both jaws. In the tiger shark the greater weight is exerted on the upper jaw, but the lateral strain appears to be taken up by the heavy, knob-like processes of cartilage on the dorsal side of the upper jaw which slide along either side of the chondrocranium near the ante-

rior part of the orbits. Tiger sharks succeed in cutting up large sea turtles—including shell into bite size pieces—a tribute to the effectiveness of their jaw mechanisms.

The majority of large species have dissimilar teeth in the upper and lower jaws, cutting teeth in one jaw and holding teeth in the other. Where teeth of the upper and lower jaws are dissimilar, only the galeoids have the cutting teeth in the upper jaw. These galeoid sharks, when attacking prey, move the upper teeth in a lateral vibratory motion. This action is clearly shown in Captain Cousteau's film "The Silent World" which shows carcharhinid sharks, probably *Eulamia* or *Carcharhinus, Pterolamiops,* and *Prionace* feeding on a whale carcass.[1] In this and similar actions, the carcharhinid shark, when opening its mouth to bite, erects the functional band of cutting teeth of the upper jaw by increasing tension of the ligament to which the teeth are attached. The bite fixes the spike-like teeth of the lower jaw in positon, and by the alternate flexing of the pectoral fins the upper part of the head and body is moved back and forth, imparting an oscillating movement to the band of teeth of the upper jaw. Lateral strains on the saw-like teeth are taken up by a pair of heavy processes of the median portion of the upper jaw and the upper jaw is protruded somewhat as the cut deepens. A portion of the large prey is thereby effectively cut off into a piece of a suitable size for swallowing.

The functional significance of differences in jaw structure and dentition in sharks of the suborders Notodanoidea and Squaloidea, in contrast to the sharks of the suborder Galeoidea, is not apparent. Observations on feeding by large notodanoids and squaloids have not been made. Large species of these suborders are restricted to cooler waters and generally to deep waters. These sharks have cutting bands of teeth in the lower jaws and holding teeth in the upper jaw. The processes of the upper jaws, when present, are flattened rather than knob-like and are located closer to the angles of the jaws than would be expected if the processes are homologous in these sharks and the galeoids.

Two pairs of long and rather slender processes of the pterygoquadrate are present in the suborder Pristiophoroidea, the sawsharks. The anterior or median pair approximates the positon of cartilages which apparently strengthen the protruded jaws against lateral strain in the carcharhinid sharks of the suborder Galeoidea, whereas the posterior pair corresponds in position on the jaw to processes characteristic of the Squaloidea. No detailed information on feeding habits of the sawsharks is available, but it appears reasonable to conclude that these processes are useful in strengthening the upper jaw when it is extended.

The foregoing summarizes, in a cursory way, much of what can be said about the relationships of structures of the jaws of sharks to their observed usefulness to the shark in feeding. I cannot, in good conscience, drop the subject without additional comment.

Limited information shows that some sharks utilize more than one class of food, including some food for which jaws and teeth are ill-adapted. The sandbar shark, *Eulamia milberti,* feeds primarily on small fishes, crustaceans, or cephalopods which can be swallowed entire; generally the fishes found in their stomachs show little evidence that the rather formidable teeth of the sandbar shark have been used at all. The whale shark, *Rhincodon typus,* feeds on plankton or on small schooling fishes; it appears to be structurally well-equipped for such fare by having a huge terminal mouth, almost rudimentary teeth, and a seive-like arrangement of gill rakers. But the whale shark also feeds, fortuitously perhaps, on tuna that are caught by an almost incredible pumping technique which takes advantage of the impetuous feeding rushes of the tuna (Gudger, 1941; Springer, 1957). Dozens of more or less similar examples could be cited: tiger sharks feeding on heavy-shelled molluscs, silky sharks feeding on fingerling-size fishes, white tip sharks skimming the

[1] Photographs of some of the sharks also shown in "The Silent World" accompany an article by Captain Jaques-Yves Cousteau: *Calypso* explores for underwater oil. National Geographic Magazine, 108:155-184.

surface with their jaws held open either as a scoop or trap (Bullis, 1961), spinner sharks feeding on shoals of fishes at the surface by spinning upward through them with the mouth open, and so on. In these examples the special structural features of the jaws and dentition are not important aids to the shark in its feeding problem.

Insufficient information precludes any precise assessment of the importance of auxiliary or substitute modes of feeding to large sharks, but the frequency of the use of one or more such methods suggests that they may be essential in the survival of many existing species. Even in such species as the sandbar shark, where the large cutting teeth of the upper jaw seem to be used rarely, the ability of the species to prey successfully on larger creatures than can be swallowed entire may be important to species survival.

Observations of living examples do not support the view that sharks use their jaws and teeth either for defense or specifically for killing prey. Some species have fin spines or armored skins which are primarily defensive. The armed rostrum of the sawshark, *Pristiophorus,* and the long upper caudal lobe of the thresher shark, *Alopias,* are the only structures of present day sharks functioning specifically for killing or immobilizing prey. The so-called fighting scars frequently noticeable on large sharks, appear on females to be the result of courtship activities, or more rarely on both sexes to be the result of accident. The principal predators on sharks are other sharks; and with the incidental exceptions mentioned, aggression of one shark upon another seems to occur only when the purpose of the aggressor is to eat the other shark. The capabilities of shark jaws and teeth for lethal action are so great, however, that segregation habit patterns which have been developed in the group probably are beneficial. These patterns have the effect of protecting a shark against larger members of its own species. Sharks in general appear to make rather precise discrimination of bulk and to evade creatures larger than themselves. But many kinds of large sharks do form schools; these schools are normally segregated by size, or sex, or both. The geographical or habitat location of nursery grounds seems to be an effective means for isolating the young of some species from their predatory parents (Springer, 1960).

Study of the evolutionary development of elasmobranch fishes has proceeded without much background for consideration of the probable adaptive value of structural innovations. This was inevitable, because the information based on the general natural history is not comprehensive. Elasmobranchs have been remarkably stable in the evolutionary sense over a long period, so that the study of living forms does not necessarily provide a fitting frame of reference for consideration of shark habits in earlier geologic periods when evolutionary processes were more actively affecting sharks. To sharks, the survival value of adequate feeding mechanisms in the face of modern competition is undoubtedly important, but examination of the shark's competitive position suggests two attributes of much greater significance. Its simple and versatile digestive system, together with its fat storage arrangements, permits a wide choice of food and allows the shark to survive long fasts between periods of gluttony. Secondly, its success in the development of various methods for producing comparatively large young is unquestionably an important reason that sharks continue to inhabit the seas.

Concerning feeding mechanisms of many existing large galeoid sharks, it might also be said that they are adequate only because their functions have been supplemented by modifications of habit, and these make the adjustments necessary in modern competition.

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# MAJOR ADAPTIVE LEVELS IN THE EVOLUTION OF THE ACTINOPTERYGIAN FEEDING MECHANISM

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## INTRODUCTION

In the higher bony fishes or Osteichthyes, feeding and gill ventilation are accomplished by movements of the visceral skeleton, the shoulder girdle, and frequently also the neurocranium. The mechanism of gill ventilation has remained relatively constant throughout the history of these fishes. Most of the modifications in the splanchnocranium have been related to the dynamics of feeding.

Of the three major groups of osteichthyans (the crossopterygians, dipnoans, and actinopterygians), the primitive actinopterygian showed by far the greatest potential for adaptive change in the feeding mechanism. Except for the skull modifications in the rhipidistian-tetrapod transition, there was but one major shift in the crossopterygians—from primitive rhipidistian to coelacanth. This involved changes somewhat comparable to the first important transformation in the actinopterygian feeding mechanism (see Jarvik, 1954, Figs. 23B and 15). The characteristic architecture of the dipnoan skull, with the palatoquadrate fused to the braincase, was established in the early Devonian, and the feeding mechanism has remained essentially unchanged since that time.

In the main stream of actinopterygian evolution from palaeoniscoid to acanthopterygian, there has been a progressive improvement in a fundamentally predaceous feeding mechanism (Fig. 1). Food was probably first obtained by biting and was swallowed whole with the aid of simple, conical pharyngeal teeth. As this biting mechanism was modified and perfected through time, the potentiality for adaptive radiation in the entire feeding mechanism increased. At the lowest or palaeoniscoid level the radiation was conservative and restricted. The changes leading to the next or holostean level improved the basically predaceous feeding mechanism and gave rise to a number of specialized types. The rise of the teleosts from one group of holosteans involved at least two different sorts of feeding mechanisms, the clupeid and elopid. Although the phylogeny of the teleosts is poorly understood, it seems evident that the specialized clupeid type was off the main line. A conservative, predaceous elopid-like stock may well represent the base for the explosive teleost radiation that began sometime in the Jurassic (Gardiner, 1960).

## THE PALAEONISCOID LEVEL AND THE BASIC ACTINOPTERYGIAN FEEDING MECHANISM

In the earliest actinopterygians, the angle of the jaw suspensorium varies from very oblique to nearly vertical. The maxillary bone, which is firmly fixed to the preopercular and infraorbital bones, forms the outer wall of a narrow chamber that contained the major portion of the adductor mandibulae muscle (Stensiö, 1921; Watson, 1925; Nielsen, 1942). The median, posterior, and ventral walls of this chamber are formed mostly by the palatoquadrate. The chamber may be open dorsally, suggesting the situation in the higher actinopterygians, or it may be closed by a median lamina of the preopercular (Fig. 4A). The palatoquadrate was movably attached to the braincase in the ethmoid region, at the basipterygoid process, and posteriorly through the hyomandibular, with which it presumably had an extensive ligamentous attachment. The mandible, which varies in length according to the angle of the suspensorium, has a nearly horizontal dorsal border and a large Meckelian fossa for the insertion of the adductor mandibulae muscle.

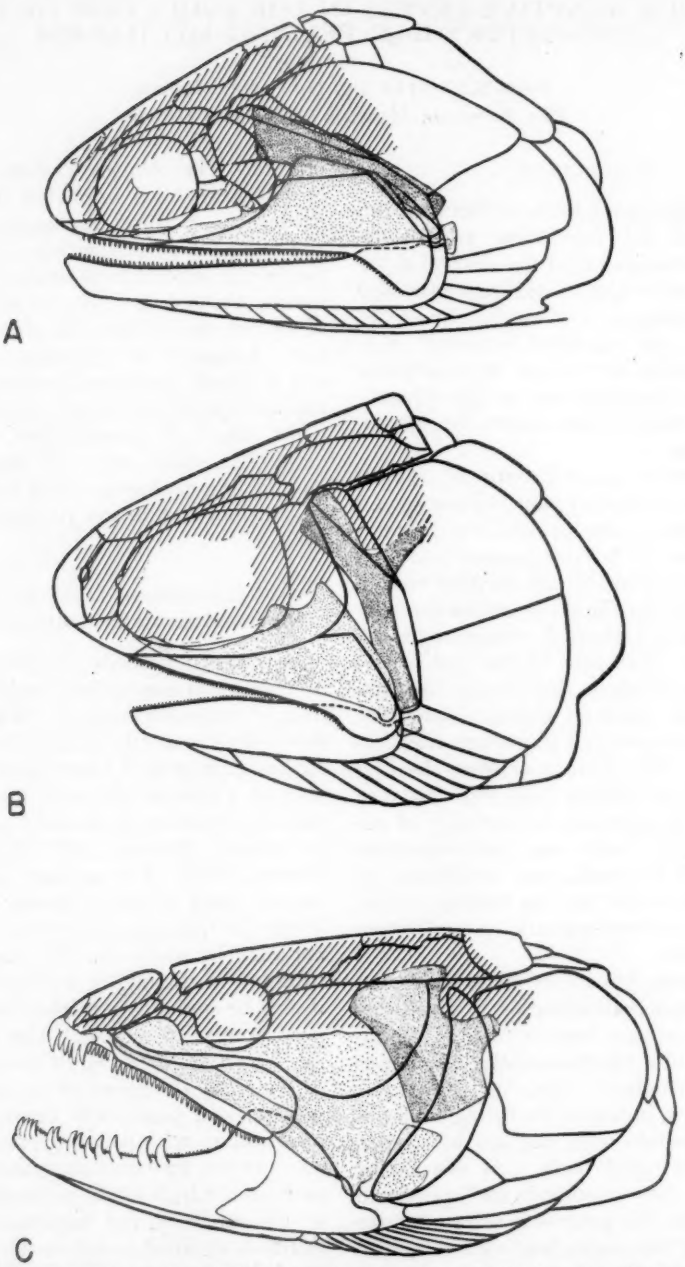



FIG. 1. Series of actinopterygian skulls from palaeoniscoid to acanthopterygian, showing changes in the angle of the jaw suspension and modifications in the palate and jaws. Each example illustrates a structural grade or level; a real evolutionary sequence is not implied. A. Palaeoniscoid (*Pteronisculus*, based on Nielsen, 1942); B. Subholostean (*Boreosomus*, after Nielsen, 1942); C. Holostean (*Amia*);

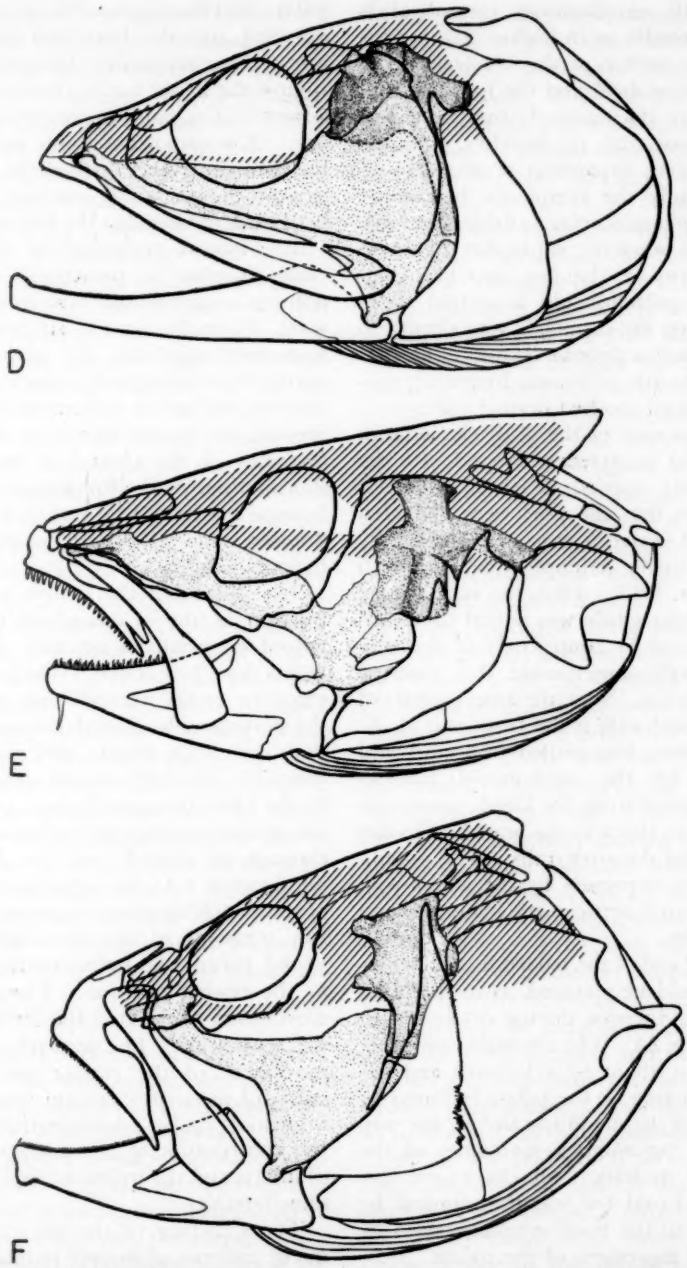



FIG. 1 cont. D. Early teleost (composite of *Notelops* and *Elops*); E. Subacanthopterygian (*Gadus*); F. Acanthopterygian (*Epinephelus*). *Gadus* is representative of a rather specialized group of teleosts sometimes aligned with the acanthopterygians. Its jaw mechanism is structurally and functionally transitional between D and F. Braincase: diagonal shading; palate and symplectic: open stippling; hyomandibular: close stippling.

The partly cartilaginous ceratohyal is flattened laterally as in higher actinopterygians. The nature of the connection between the ceratohyal and the hyomandibular is poorly documented, but in a few forms, at least, the (?) interhyal and the symplectic were apparently arranged in series, with only the symplectic in contact with the hyomandibular. Aside from the presence of separate suprapharyngobranchial elements in the first two branchial arches, the palaeoniscoid branchial skeleton, including the copula, is very similar to that of higher actinopterygians. The oral surface of the gill arches was frequently covered with small toothed dermal plates.

The disposition of the skull elements in palaeoniscoid specimens preserved with the mouth widely opened (Nielsen, 1942, Pl. 13) indicates that the jaws were abducted and visceral skeleton expanded essentially as in most living actinopterygians (Fig. 2) (Tchernavin, 1953). First, the ventral part of the shoulder girdle was pulled backward and downward by contraction of the ventrolateral body musculature that inserted on the cleithrum. Next, the anterior part of the copula and with it the front end of the hyoid bar were also pulled backward and downward by the sternohyoid muscles which extended from the cleithrum to the hyoid bar. In this way the branchial basket was expanded dorsoventrally. The mandible was then depressed by the geniohyoid muscles situated between the hyoid bar and the mandible.

The front end of the palaeoniscoid neurocranium could be elevated, as in many recent fishes, to increase the size of the mouth opening (Fig. 2A). The elevation was probably brought about by a forward and upward force acting on the palate initiated by depression of the mandible and by the contraction of the anterior myotomes of the dorsolateral muscles. The backward motion of the hyoid bar was thus limited by attachment to the fixed symplectic and by the forward movement of the palate.

These conflicting forces caused the posterior end of the hyoid bar to swing laterally. As a result, the mandibular rami, the palate, and the hyomandibular were pushed outward and the branchial basket consequently was expanded. The principal function of the hyoid bar is, therefore, to act as a lever that expands the orobranchial chamber. The importance and uniqueness of this function (see Tchernavin, 1953) may explain the essentially constant form of the hyoid bar throughout the Osteichthyes.

The lateral expansion of the orobranchial chamber in palaeoniscoids with an oblique suspensorium was limited in two ways. First, the vertical articulation of the hyomandibular with the braincase would permit it to swing only anteriorly, thereby limiting the lateral movement of the palate. Second, the lateral excursion of the posterior end of the ceratohyal was probably shorter than in the holosteans and teleosts because the ceratohyal was tied to the mandibular arch rather than separately to the hyomandibular.

The closing of the mouth and the contraction of the orobranchial chamber involved essentially a reversal of the movements described above. When the anterior segments of the ventral body muscles and the sternohyoids relaxed, the tension on the shoulder girdle, copula, and mandibles was removed. With the mouth closed and fixed by the adductor mandibulae, contraction of the geniohyoids would pull the copula and, through the sternohyoids, the shoulder girdle forward. At the same time, adduction of the mandible and relaxation of the anterior segments of the dorsolateral muscles would permit the neurocranium to return to the resting position. These combined movements eliminated the forces acting on the ceratohyal. The forward and upward movement of the copula and the down swing of the neurocranium compressed the branchial basket dorsoventrally, whereas the constriction of the adductor hyomandibularis and the intermandibularis did the same laterally.

The structure of the jaws and the frequent presence of sharply pointed teeth suggest that most palaeoniscoids were principally predators. The deep gape and the shallowness of the orobranchial chamber

with the mouth widely opened further suggest that food was usually obtained by biting rather than by filtering or by engulfing through current action. Biting is simply seizing food with the jaws or teeth. A small piece may be cut from a larger mass, or small prey may be seized and gulped whole. Larger, entire prey may also be obtained by seizing, releasing, swimming forward and seizing again—a sequence repeated until the entire prey is within the orobranchial chamber (Breder, 1925). Any of these ways may have been employed by the palaeoniscoids, depending in part on the length of the jaws and perhaps on the obliquity of the suspensorium.

The force of the bite is directly related to the mass and disposition of the adductor mandibulae muscle. In the palaeoniscoids the adductor was confined to the narrow palatoquadrate-maxillary chamber (Figs. 3 and 4A), from which it passed into the Meckelian fossa immediately anterior to the jaw articulation. Functionally, the mandible is a straight lever with a very short effort arm. Obviously the palaeoniscoid biting mechanism was efficient enough for these fishes to survive for roughly 200 million years. Compared with the holostean condition, however, the palaeoniscoid adductor was less powerful and the torque about the jaw articulation far below that in forms with a coronoid process on the mandible. Also, the expansion of the orobranchial chamber and even the depression of the mandible were less effectively accomplished at this level than at the holostean.

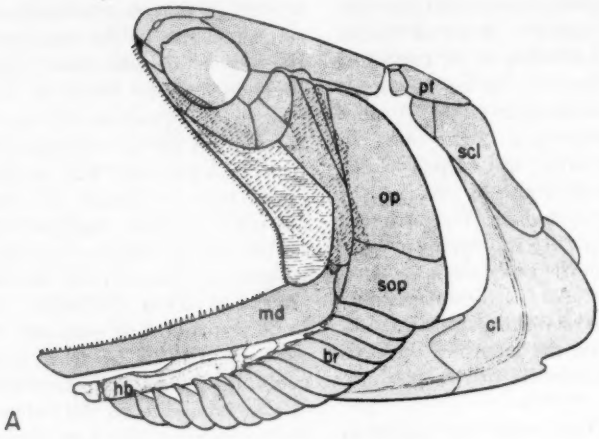
### THE PALAEONISCOID–HOLOSTEAN TRANSITION

Throughout the history of the palaeoniscoids there was some experimentation in the feeding mechanism, mostly related to the obliquity of the suspensorium.

In a few groups of palaeoniscoids (Westoll, 1937, 1944), and in a heterogeneous assemblage of advanced chrondrosteans called subholosteans (Fig. 1B), the suspensorium is nearly vertical. The dorsal border of the maxillary-palatoquadrate chamber is open, and in the subholosteans, at least, the origin of the adductor mandibulae may have extended, in part, to the braincase. Assuming some increase in the mass of the adductor, the power of adduction was correspondingly increased. With the hyomandibular now swinging in a nearly vertical plane, the mechanism for lateral expansion of the orobranchial chamber was improved. In this connection, it should be emphasized that the palaeoniscoids and particularly the subholosteans approached the holostean level in various characters of the skull and postcranial skeleton (Schaeffer, 1956). There is now substantial evidence that this level was attained by at least three separately evolving palaeoniscoid-subholostean lines.

The final shift to the holostean grade occurred during the late Permian and the early Triassic. It involved a number of inter-related changes in the jaw mechanism that followed acquisition of a vertical suspensorium. The most important of these was the elimination of the maxillary–palatoquadrate chamber. This was brought about by separation of the maxillary from the preopercular and the infraorbital bones, by a reduction in the width of the preopercular and separation of it from the dermal elements behind the orbit, and by modifications in the shape, size, and curvature of the dermal components of the palatoquadrate (Figs. 3B and 4B). The hyomandibular became relatively shorter and broader and more closely associated with the palate, and the upper border of the mandible was elevated posteriorly as the coronoid process.

The opening of the cheek region and the changes in the palate permitted further expansion of the adductor mandibulae muscle (Fig. 3). In the evolutionary line that gave rise to the amioids and independently to the pholidophoroids (which, in turn, produced the teleosts), the adductor muscle, as in *Amia*, must have extended its origin to include the postorbital part of the braincase, the adjacent ventral surface of the skull roof, the posterior part of the palate and the hyomandibular, and the entire anterior border of the preopercular. With this expansion, the muscle increased in mass (volume and number of fibers) and became distinctly subdivided. Although the major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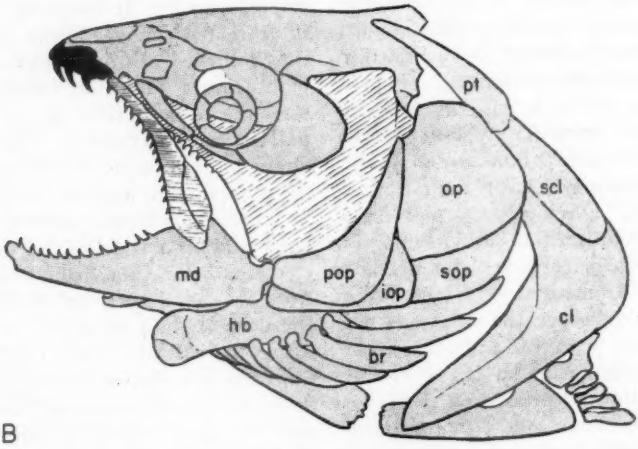
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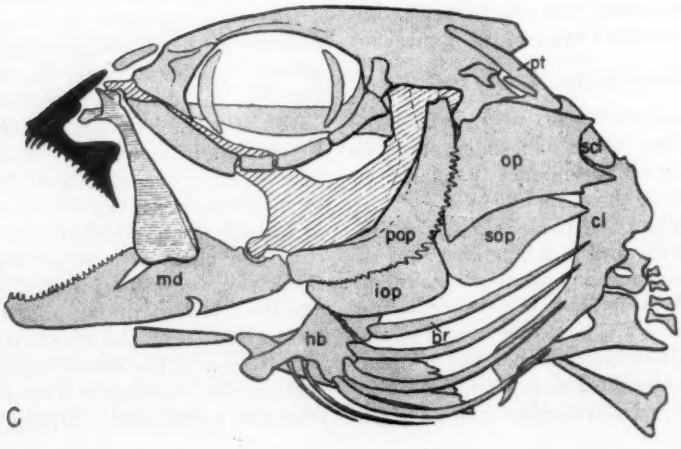
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FIG. 2. The actinopterygian skull with the mouth widely opened. In A. (*Pteronisculus*), the palaeoniscoid maxilla is fixed to the cheek, the braincase elevated, and the shoulder girdle moved backwards. In B. (holostean and sub-acanthopterygian condition represented by *Salmo*), the maxilla is swung forward as the mandible is depressed, the braincase frequently elevated, and the shoulder girdle moved backward and downward. In C. (the acanthopterygian *Centropristes*), the same motions occur, but in addition the premaxilla is projected forward. Note reduction in length of hyoid bar and number of branchiostegals (see footnote 1). Palate and hyomandibular: obliquely lined; maxilla: horizontally lined; premaxilla: solid black. Palaeoniscoid palate and hyomandibular as seen through cheek: oblique broken lines. Abbreviations: br, branchiostegals; cl, cleithrum; hb, hyoid bar; iop, interopercular; md, mandible; op, opercular; pop, preopercular; pt, posttemporal (suprascapular); scl, supracleithrum; sop, subopercular.

portion of the muscle still inserted within the Meckelian fossa as in the palaeoniscoids (and as in *Amia* and most teleosts), one subdivision became attached to the posterior border of the coronoid process (Fig. 4).

The resulting increase in the absolute power of the adductor mandibulae obviously augmented the strength of the bite. The disposition of the muscle in *Amia* further indicates that its subdivision and extensive origin permits at least one part to exert maximum pull on the mandible throughout the adduction cycle. Regarding the mandible as a lever, it is apparent that the force is applied immediately in front of the fulcrum, and that the effort arm is very short compared with the resistance arm regardless of whether the coronoid process is present or absent. Assuming the resistance arm (dentigerous portion) to be of the same length in a palaeoniscoid and a holostean, the torque about the articulation will be greater in the latter simply because the magnitude of the force acting on the mandible is greater. But more than that, the elevation of the coronoid process transforms, in part, the straight lever of palaeoniscoid type into a bent lever. The straight-lever analogy still applies in the holosteans and teleosts to that portion of the mandibular ramus anterior to the coronoid, with the effort applied by the subdivisions of the adductor entering the Meckelian fossa. The bent lever refers to the coronoid process, with the effort applied by the adductor subdivision inserting along its posterior slope.

The adaptive significance of the coronoid process, which is slightly developed even in a few palaeoniscoids and is variously elevated in most of the higher actinopterygians, is perhaps best explained in terms of torque. For purposes of analysis, it may be assumed that the force is applied only at the apex of the process and at right angles to its posterior border. If the torque value is regarded as unity in the palaeoniscoids where the coronoid process is usually absent, progressive increase in the height of this process, other things being equal, results in a geometric increase in torque.

The probable modification of the uppermost palaeoniscoid branchiostegal ray into the holostean interopercular bone was associated with a new way of depressing the mandible. As van Dobben (1937) has demonstrated, contraction of the levator operculae muscle will elevate the entire opercular series and through the interopercular–mandibular ligament (which is attached to the mandible below and behind the jaw articulation), depress the lower jaw. The interopercular serves to converge the opercular series on the ligament (Fig. 2).

Aldinger (1937, p. 332) and Nielsen (1942, p. 187 and p. 350) have pointed out that the location of the hyomandibular articulation on the median face of the opercular bone would affect the ability of the dilator operculi muscle to abduct the opercular and subopercular. When this articulation is situated near the mid-point on the anterior border of the opercular, as in *Pteronisculus* and probably most other palaeoniscoids with an oblique suspensorium, the action of the dilator operculi is more limited than when the articulation is close to the anterodorsal corner of the opercular bone, as in *Amia* and most teleosts. The firm connection of the hyomandibular with the palate and the overlying opercular elements, including the subopercular, suggests that wide abduction of the gill cover oc-

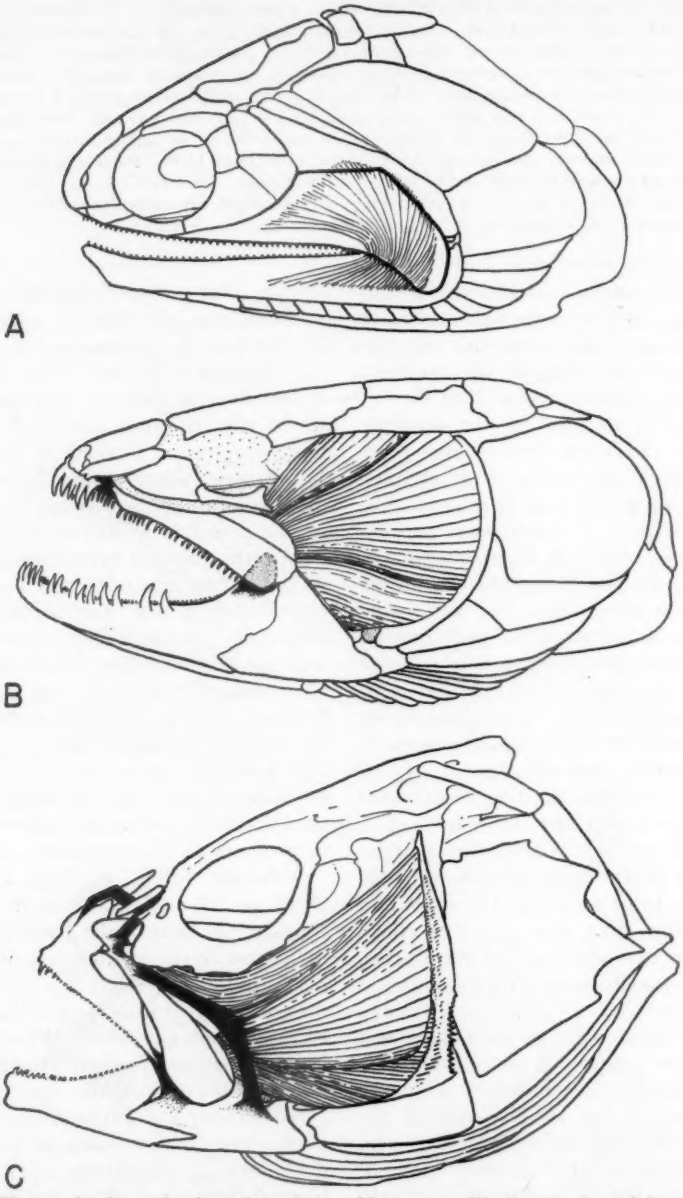



FIG. 3. Changes in size and orientation of the adductor mandibulae muscle and jaw ligaments in forms representing three major adaptive levels of the actinopterygian feeding mechanism. A. Palaeoniscoid (*Pteronisculus*), showing the probable extent of the adductor mandibulae in the maxillary–palatoquadrate chamber, as seen through the dermal bones of the cheek. The maxilla, heavily outlined, was probably without a specific ligament to the mandible. B. Holostean (*Amia*), with the postorbital bones removed to show the superficial division of the muscle, with its three components. Areas occupied by ligaments solid black, except where the maxillary-mandibular ligament extends underneath the maxilla toward the coronoid process of the mandi-

ble (darkly stippled). C. Acanthopterygian (*Epinephelus*) where the superficial division of the adductor mandibulae has been reduced to two components, both of which primarily influence the behavior of the maxilla. The now protrusile premaxilla is extensively braced by rostral ligaments.

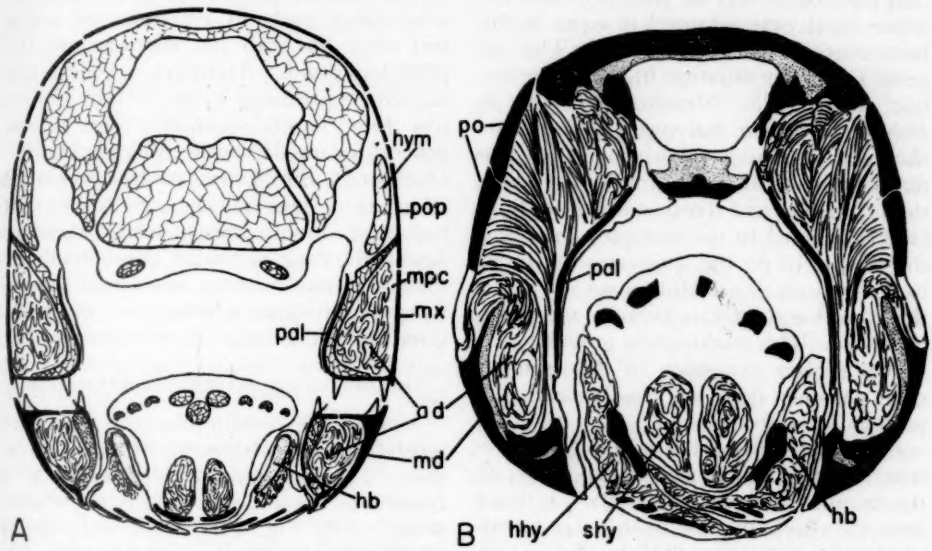



FIG. 4. Diagrammatic transverse sections of the palaeoniscoid and holostean head. A. *Pteronisculus* (partly after Nielsen, 1942). B. *Amia*. Note that the upper portion of the adductor mandibulae muscle is confined to the maxillary-palatoquadrate chamber in A, and that the absence of the chamber in B is correlated with the greater mass of this muscle. Section A is anterior to the passage of the adductor into the Meckelian fossa; section B is behind the apex of the coronoid process. Abbreviations: ad, adductor mandibulae muscle; hb, hyoid bar; hhy, hyohyoideus muscle; hym, hyomandibular; md, mandible; mpc, maxillary-palatoquadrate chamber; mx, maxilla; pal, palate; po, postorbitals; pop, preopercular; shy, sternohyoideus muscle.

curred only during expansion of the orobranchial chamber.

In palaeoniscoids and subholosteans with a vertical suspensorium, the relationship of the hyomandibular to the palate was clearly less binding. With a more dorsal position for the hyomandibular-opercular articulation, contraction of the dilator operculi could presumably abduct the opercular series as in the holosteans and teleosts. Closing of the gill cover must have been accomplished by an adductor operculae.

The levator operculi, representing a third dorsal subdivision of the original hyoid constrictor, may not have been fully differentiated from the adductor below the holostean level. There may have been ligamentous connections between the upper few branchiostegal rays and the mandible (*Amia* still has a ligament between the branchiostegal below the interopercular and the mandible) to tie these elements together at the point where the branchiostegal series "turns the corner" to the throat region. The branchiostegals provide an elastic cover for the opercular chamber where the latter is associated with the hyoid bar and the mandible (Woskoboinikoff, 1932).[1] With the subopercular tied to the hyomandibular as in *Pteronisculus*, elevation of the opercular series would be nearly impossible. In forms with a vertical suspensorium, this connection was certainly no more than ligamentous (as in *Amia*) and some vertical move-

[1] At the paleoniscoid level, the number of branchiostegal rays and the length of the hyoid bar are related, in part, to the length of the mandible and obliquity of the suspensorium.

ment was perhaps possible. Until differentiation of the interopercular bone, however, this movement could not be focused on the mandible.

It is probable that the symplectic and the (?)interhyal were arranged in series in the palaeoniscoids and subholosteans. This appears to be the situation in the palaeoniscoid *Acrorhabdus* (Stensiö, 1921) and in *Polyodon*. In the holosteans and teleosts the interhyal is attached directly to the hyomandibular behind the symplectic. With the broadened and shortened hyomandibular firmly fixed to the metapterygoid, and the symplectic to the quadrate, the interhyal functions as a mobile pivot on which the hyoid bar can rotate laterally and swing backward. This modification probably permitted greater expansion of the orobranchial chamber than was possible with the palaeoniscoid arrangement.

In the palaeoniscoids, the labial fold presumably fitted snugly around the corner of the mouth. When the maxilla was freed from the cheek, the fold in this area broadened and became more flexible. A new ligamentous attachment developed within the fold between the posterior end of the maxilla and the coronoid process (Fig. 3A, B). Thus when the mandible was depressed, the maxilla was pulled anteriorly, pivoting at the ethmopalatine articulation. In this way the maxillary dentition was carried forward to increase the number of teeth immediately available for grasping prey, while the stretching of the labial fold behind the maxilla extended the orobranchial chamber forward.

### THE HOLOSTEAN LEVEL

All these modifications leading to the holostean level greatly increased the opportunity for adaptive radiation in the feeding mechanism. Within this level, the independently derived semionotids developed short, powerful jaws and in many genera a crushing dentition. The long-snouted lepisosteids, or gars, presumably evolved from the semionotids, represent a radical departure from the ancestral skull pattern. The Mesozoic pycnodonts, which arose from equally deep-bodied palaeoniscoids and subholosteans, lost the maxilla and had the skull otherwise altered in conjunction with an extremely specialized crushing dentition.

The conservative, early Triassic parasemionotids probably represent the ancestral stock for both the amioids and the pholidophoroids (Gardiner, 1960). The amioids, represented today only by the genus *Amia*, mostly retained a more or less generalized, predaceous feeding mechanism. One family, the Macrosemiidae, developed short, strong jaws like the semionotids, but had long, sharply pointed teeth. Another family, the Pachycormidae, questionably retained in the Amioidea, developed a heavily ossified rostrum which in one genus became elongated, as in the swordfishes.

### THE HOLOSTEAN-TELEOSTEAN TRANSITION

Among the pholidophoroids there are several families with specialized jaw structure. The central stock, represented by a form like *Pholidophorus similis* (Saint-Seine, 1949, Fig. 94), shows only slight modification of the basic predaceous pattern. One character of importance is the reduction of the dorsal process of the premaxilla. The premaxilla in the palaeoniscoids and subholosteans is a small element with no indication of a dorsal process. Apparently it is frequently fused with other rostral bones, and its origin in the holosteans is obscure (see Nielsen, 1942, p. 137-140). In the semionotids and the amioids, the dorsal process is large and firmly fixed to the ethmoid part of the neurocranium. This was presumably the situation in the Parasemionotidae. The reduction of the dorsal process in the pholidophoroids and particularly in the Pholidophoridae which gave rise to the teleosts, suggests that the premaxilla may have been slightly mobile as in the isospondylous teleosts.

Although detailed studies of the pholidophoroid visceral skeleton have not yet been made, available evidence (Rayner, 1948; Saint-Seine, 1949) supports the conclusion that the holostean feeding mechanism, as described above, remained essentially unchanged during the transition to

the teleost level. The significant advances here were probably related to improvement in locomotion. Coupled with the particular design of the pholidophoroid skull, the stage was set for the explosive teleost radiation.

### THE EARLIEST AND SUB-ACANTHOPTERYGIAN TELEOSTS

By the late Triassic, the leptolepids, usually regarded as the first teleosts, had arisen. Their small gape and short, deep mandible suggests that they gave rise only to the clupeids and closely related groups. The main line of teleost evolution, which preserved the basic predaceous feeding mechanism, probably extended independently and through unknown Jurassic forms from the pholidophoroids to the elopids. Aside from the leptolepids (and the related lycopterids), the elopids are among the few known early Cretaceous teleost groups. There was thus a lag in the teleost radiation throughout the Jurassic, perhaps related to holostean competition. The great teleost diversification, stemming mostly from elopid-like stock, began early in the Cretaceous or perhaps in the late Jurassic. It is possible that many of the *families* of modern teleosts, including the acanthopterygian, were in existence by the close of the Cretaceous.

The major modifications in the teleost feeding mechanism during and after the Cretaceous were mostly confined to the palate and jaws.

In Cretaceous elopids such as *Notelops* (Dunkle, 1940), perhaps the earliest known predaceous teleosts,[2] and in the Recent genus *Elops* (ten pounders), many primitive characters such as a gular plate, vertical jaw suspension, postorbital lower jaw articulation, maxillary teeth, and extrascapular bones were retained (Fig. 1D). In other characters there was a marked advance. The palate probably became less well ossified as did many of the dermal bones, and the upper jaw clearly underwent specialization. The premaxilla and maxilla, which in *Amia* are arranged essentially in series, in elopids are overlapped. The overlapping or tandem arrangement of these bones, beginning in the pholidophoroids, resulted from the extension of the premaxilla posteriorly along the inferior edge of the maxilla, and from the extension of the upper end of the maxilla inward toward the premaxillary symphysis.

In elopids, apparently for the first time, the maxilla and palatine are joined by a ball and socket hinge, in contrast to the simple connective tissue hinge of the holosteans. In *Elops* the maxilla has a small bony nubbin on its inner dorsal aspect that is seated within a corresponding palatine excavation; the expanded club-shaped tip of the maxilla is joined by connective tissue to the ethmovomer block. The dorsal process of the premaxilla, well developed in most holosteans, is represented in elopids by a small, pointed elevation that articulates with the ethmoidal cartilage but which is no longer locked in position as in *Amia*. All of these modifications foreshadowed a new mobility for the upper jaw, although the symphyseal portion of the premaxilla was still held firmly to the rostrum by inelastic connective tissue.

Because of its anterior position, the premaxilla is the principal biting component of the upper jaw and the first to contact the prey. Its toothed ramus was gradually extended as a narrow strut below and parallel with the maxilla, excluding the latter from the gape of the mouth and functionally replacing it.

The adaptive modification of the premaxilla created a mechanical problem for the movable maxilla. As the maxilla rotated forward it must have pressed against the toothed ramus of the premaxilla even in the first stages of premaxillary enlargement. The original accommodation of the premaxilla to maxillary motion probably necessitated complex changes. In holosteans (except possibly in the pholidophoroids) the premaxilla is locked in position by the dorsal process, and presumably this process had to be lost before premaxillary movement was possible. The original bony

[2] See above, regarding our views on the relationships of the leptolepids.

contact of the premaxilla with the rostrum may have been replaced by a more flexible one of cartilage and connective tissue even in the earliest teleosts.

Growth backward of the toothed ramus of the premaxilla into the gape of the mouth and increase in the flexibility of the rostral premaxillary connection are mutually re-enforcing changes. The longer the toothed ramus of the premaxilla, the more maxillary movement is translated to it, and the greater is the need for a flexible dorsal connection in which the premaxilla can rock back and forth. Conversely, more premaxillary movement is possible as the bed of rostral connective tissue becomes more extensive, and thus more opportunity is afforded to increase the length of the premaxillary ramus. Some of the argentinids, close relatives of the smelts, provide excellent examples of these interrelated changes carried to their fullest extent.

### THE ACANTHOPTERYGIAN LEVEL

The evolution of the highly kinetic acanthopterygian jaw mechanism from a relatively immobile isospondylous type apparently involved (i) a progressive shift forward of the palatoquadrate arch and the resultant forward movement of the lower jaw articulation, (ii) freeing and further enlargement of the premaxilla and exclusion of the maxilla from the gape of the open mouth, (iii) consolidation and simplification of the subdivisions of the mandibular muscles, (iv) insertions of one or more divisions of the mandibular muscles (usually the external one) on a tendon to the maxilla,[3] and (v) the origin of new ligaments from the maxilla to the ethmoid and/or palatine bone, and from the upper end of the premaxilla to the palatine (Fig. 3C). The key to all of these changes appears to have been the initial freeing of the maxilla so that it could be pulled downward and forward by the lower jaw as the mouth was opened.

The movable suspension of the maxilla from an ethmopalatine articulation is an important feature of early teleost predaceous jaw mechanisms. Later, the movement of the maxilla was to encourage indirectly the development of a system of ligaments controlling the premaxilla, as in the predaceous acanthopterygian jaw. The acanthopterygian type, which incorporates a protrusile *premaxilla,* enables the fish to project its upper jaw toward food (Fig. 2C) with great rapidity while extending the orobranchial chamber further forward than was possible by maxillary movement alone.

The predaceous mouth usually requires a solid footing for the upper jaw: the premaxilla not only must be able to resist the impact of hitting prey with force but must also provide a solid foundation for the lower jaw to snap closed against. Thus, in some of the more generalized acanthopterygian predators such as serranids (sea basses) and carangids (jacks), the premaxilla is held rather firmly to the rostrum and is only slightly protrusile. In such highly predaceous forms as the barracuda (*Sphyraena*) and pike killifish (*Belonesox*), both specialized descendents of fishes with strongly protrusible mouths, the premaxilla has secondarily lost its mobility to modifications that increase the strength and rigidity of the upper jaw. Hence, even the predaceous acanthopterygian mouth, like that of base-line palaeoniscoids, holosteans, and early teleosts, remains primarily a biting or snapping mouth, although the seizing mechanism has been improved significantly.

Even though the protrusile premaxilla only slightly increases the overall effectiveness of the predaceous mechanism, it greatly increases the evolutionary potential of the mouth parts. It is primarily the acanthopterygian mouth that has given rise to

[3] In *Amia* a component of the external division of the adductor mandibulae inserts partly on a ligament that extends from the upper third of the maxilla to the coronoid process of the mandible. Possibly this component with its ligamentous attachment to the maxilla and coronoid represents the muscle primarily involved in maxillary movement in the acanthopterygian teleosts. In serranids, at least, this ligament has a connection with the maxilla and mandible, although the mandibular section is much reduced and is absent in other percoids.

the enormous variety of specialized predaceous *and* non-predaceous feeding mechanisms for which the teleosts are so well known. Presumably the evolution of the acanthopterygian jaw mechanism promoted the successful exploitation of food sources that previously were largely unavailable to actinopterygian fishes. In a group of Marshallese coral reefs recently analyzed (Hiatt and Strasburg, 1960), more than 75 per cent of the resident vertebrate consumers, including such diverse feeders as primary and secondary carnivores and coral polyp, algae, and detritus eaters, belong or are related to the acanthopterygian order Perciformes. The protrusile mouth of the perciform fishes and their derivatives probably was and continues to be a major factor in their success. These dominant fishes, numbering more than 8,000 species, constitute about half of today's fresh- and saltwater ichthyofauna.

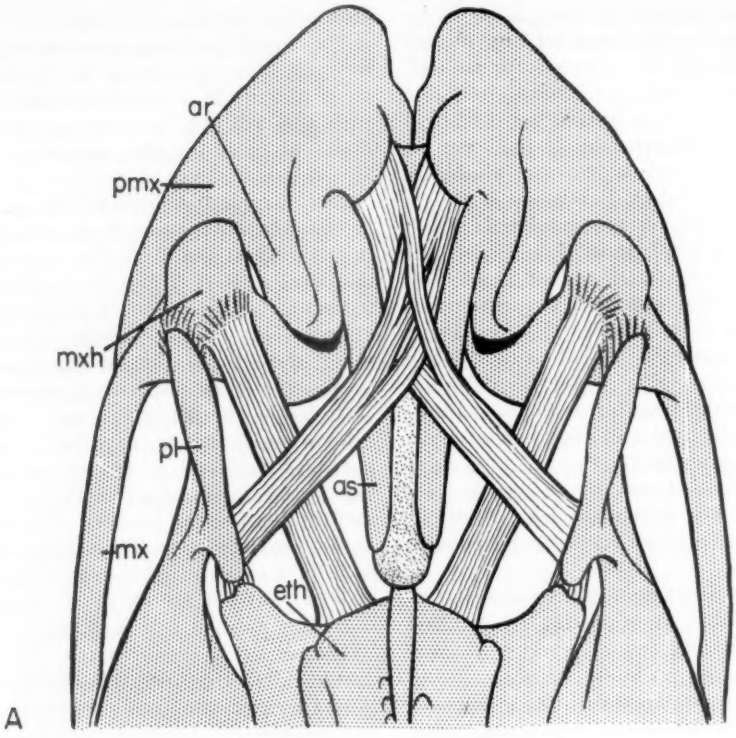
In a generalized type of protrusile upper jaw, the premaxilla is a strut-like toothed bone that extends backward and downward from the rostrum. The upper, anterior end is held loosely to the ethmoid by connective tissue. The posterior end, which stops short of the lower jaw, is joined by a ligamentous fold to the inferior edge of the maxilla. The maxilla is usually fairly long, and bowed inward at its upper end or head. On its posterior surface the head of the maxilla has a firm ligamentous connection with the palatine, and medial to this connection the maxilla has a much looser, anterior, connective tissue association with the premaxilla. The longer, posterior section of the maxilla, or arm, is joined by a ligament to the lower jaw near the coronoid process.

When the lower jaw moves downward, the arm of the maxilla is pulled downward and forward, swinging on the palatine hinge. The posterior end of the premaxilla, which is held by ligaments to the inferior edge of the maxilla, also moves forward, as does the upper end of the premaxilla, which is only gently restrained by the loose connective tissue network of the rostrum. In this manner the premaxilla slides forward until the rostral connective tissue becomes taut. This mechanism contrasts with that of the elopid, and most other sub-acanthopterygian types, in which the posterior or lower end of the premaxilla swings forward with the maxilla, but the upper end remains in place on the rostrum (Figs. 1E, 2B, and 6).

In the extended position, the protrusile premaxilla moves away from its solid footing on the rostrum (Fig. 2C). Two distinct mechanisms have arisen to brace the protruded upper jaw. One involves a modification of the head of the maxilla, and the other, the organization of the rostral connective tissues into specific ligaments.

Probably the original means of ensuring proper and continuous premaxillary support was the development of an interlocking arrangement between the premaxilla and the head of the maxilla. In many clupeids (herrings), and argentinids, the premaxilla has acquired an ascending bony process (not the holostean dorsal process), that rises upward and backward from the symphysis. The head of the maxilla has developed an anteriorly directed flange dorsally and a similar though usually smaller one ventrally. Together the two flanges form a groove into which the posterior edge of the premaxilla fits snugly when the mouth is closed. When the mouth opens, the maxillae in moving forward push the premaxillae ahead of them. The ascending premaxillary processes slide forward in a connective tissue track over the ethmoid cartilage as the maxillae rotate forward so that their grooved heads maintain contact with the premaxillae. Thus are the premaxillae braced by the maxillae during forward movement.

In the cyprinodontiforms (killifishes) and mugiliforms (mullet, silversides, barracuda), the maxilla has become further specialized to brace the protruded premaxilla. This has been accomplished by an increase in the length of the maxillary head and the bony flanges that form the maxillary groove; hence, contact between maxilla and premaxilla is maintained throughout more of the protrusion cycle. Protrusibility of the upper jaw has been enhanced, as in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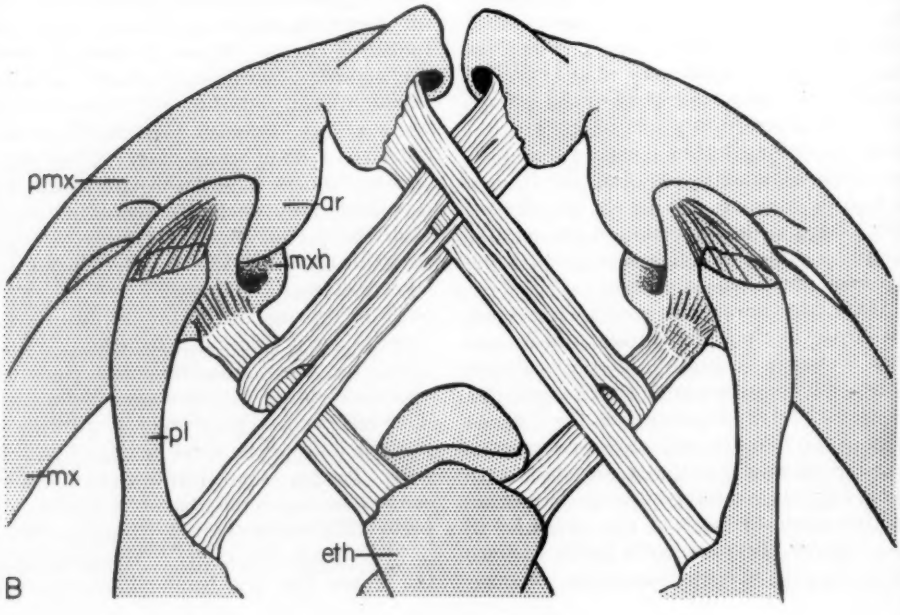
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many acanthopterygians, by the development of a direct ligamentous connection of the posterior end of the premaxilla with the lower jaw. Movement of the premaxilla forward is thus accomplished without the intervention of the maxilla. The maxilla in these fishes exercises principally a restraining and bracing function for the premaxilla.

In the generalized acanthopterygian fishes the protruded upper jaw is braced against the various stresses entailed in grasping food both by the maxillary head and by a series of rostral ligaments (Fig. 5A). The ligaments, that presumably arose through consolidation of less organized connective tissue along stress lines, form a system of cross-braces that provide strength in many directions. In general, the paired upper jaw ligaments in acanthopterygians and in certain soft-rayed teleosts with protrusile mouths when present are either crossed, that is, extending from the right to the left side of the jaw mechanism, or at least obliquely situated. The simplest and apparently fundamental arrangement of these ligaments to be found in the predaceous jaw is a pair running obliquely from ethmoid to maxilla crossed above by a pair connecting left and right premaxillary heads with, respectively, the right and left palatines. When an upper jaw so equipped is protruded, the forces pulling one premaxilla toward the other (and thus maintaining the symphysis in the median plane) increase with protrusion. Thus, the system of crossed ligaments combines multidirectional strength with a flexibility sufficient for protrusion, and represents an overall improvement of the jaw mechanism, predaceous and otherwise.

Protrusibility is not a necessary concomitant of developing upper jaw ligaments for such ligaments occur in cods and pirate-perch which have essentially non-protrusile premaxillae (Figs. 5B and 6). Protrusibility requires also the development of elaborate premaxillary processes (ascending, articular, and lateral), special maxillary-premaxillary and maxillary-palatine associations, and particularly appropriate muscle insertions (see Gregory, 1933, and Eaton, 1935). But the appearance of ligaments controlling premaxillary motion means that the maxilla is no longer solely responsible for regulating the forward excursion of the premaxilla—a function now also assumed by the rostral ligaments. This division of responsibility may have made it possible for the head of the maxilla to become modified for new roles with a minimized risk to the protrusile mechanism of the premaxilla.

The gerrids (mojarras), specialized percoids that graze on small organisms, provide an example of the maxilla in a specialized functional association with the upper jaw. The very small mouth of the mojarra is astonishingly protrusile (Fig. 7), and is thrust rapidly out and back repeatedly during feeding. The ascending processes of the premaxillae are greatly elongated so that when the mouth is closed they extend back over the ethmoid region and over the anterior portion of the frontals. The premaxillary–palatine ligaments have lost contact with the premaxillae and have become joined together as a broad band connecting right and left palatines. Under this strap-like ligament the long ascending premaxillary processes slide back and forth. The maxillae no longer act as levers to protrude the premaxillae, which now have their own independent ligamentous attachment to the lower jaw.

Abduction of the lower jaw and contrac-

FIG. 5. Semidiagrammatic representation of the upper jaw ligaments in a sea bass (A), in which the premaxilla is protrusible, and in a cod (B), in which the premaxilla is not protrusible. Note that although both fishes have essentially similar ligaments the protrusible premaxilla has an ascending process, and the non-protrusile premaxilla does not. In the sea bass the maxilla is activated by the external division of the adductor mandibulae muscle, as is usual in fishes with protrusile premaxillae. In the cod this division of the muscle is reduced or absent and the maxilla, instead, receives an internal slip of the adductor that originates on the palatoquadrate. Abbreviations: ar, articular process of premaxilla; as, ascending process of premaxilla; eth, ethmoid region; pl, palatine; pmx, premaxilla; mx, maxilla; mxh, head of maxilla.

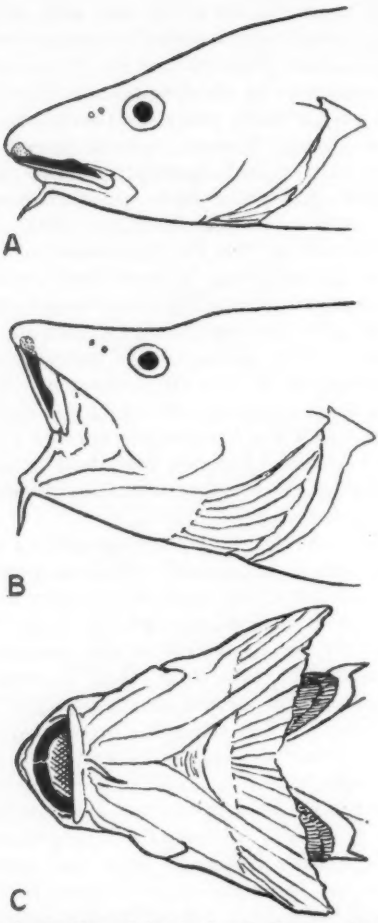


FIG. 6. Outline of head of cod (*Gadus*) showing opening of mouth, movement of the premaxilla (solid black), and expansion of orobranchial chamber. A. Side view with mouth closed. Premaxilla lies almost horizontally. B. Side view with mouth widely opened. The posterior portion of the premaxilla has been pulled forward, but the upper, anterior end has remained in place on the rostrum and this premaxilla is therefore non-protrusile. Note increase in depth of throat over the condition in A and elevation of neurocranium. C. Ventral view, showing lateral expansion of the jaws and orobranchial chamber when the mouth is widely opened. Modified from Tchernavin (1953).

tion of the external division of the adductor mandibulae muscle in the mojarra causes the maxillae to rotate *inward* approximately 90° so that their grooved heads face each other, giving the appearance of opposing parenthesis marks, thus ( ). In coming together to form this transitory, ring-like configuration, the grooved maxillary heads close around the ascending premaxillary processes in advance of the strap-like cross-palatine ligament. As the premaxillae are drawn out by the downward movement of the lower jaw, the ascending processes slide forward under the posteriorly situated cross-palatine ligament and within the roughly circular enclosure formed more anteriorly by the apposed grooves of the maxillary head. The long ascending processes of the premaxillae are supported by the maxillary track and cross-palatine ligament at all times during the forward excursion of the upper jaw, and are thus doubly supported and braced against forces that would drive them out of alignment. In summary, the maxilla of the mojarra has assumed a new role in an effective grazing mechanism as a result of having been (i) freed from its function of protruding the premaxilla by the origin of a direct ligamentous premaxillary-mandibular association, and (ii) aided in its control of premaxillary alignment by the rostral ligaments.

Paradoxically, there are teleosts in which the upper jaw is highly protrusile but lacks most or all of the typical acanthopterygian upper jaw ligaments (cypriniforms, cyprinodontiforms, mugiliforms, for example). There is a variety of reasons for thinking that such fishes may have arisen from subacanthopterygian ancestors before patterns of ligaments were stabilized. If so, each represents an early and independently successful experiment in protrusibility. But these teleost groups are exceptions that prove the rule. Combined, they produced only a limited array of specialized feeding mechanisms. The basic predaceous acanthopterygian mechanism, however, has given rise to a seemingly boundless variety. This great increase in the adaptive potential of the acanthopterygian jaw, as compared with that of predecessor types, may be attributable in some measure to the presence in the acanthopterygian mouth of

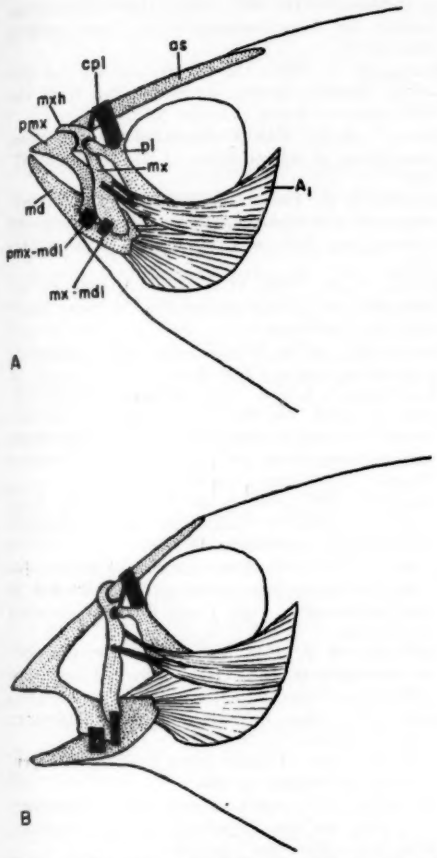


FIG. 7. Diagram representing protrusion of the upper jaw in a gerrid (mojarra). A. Mouth closed. B. Mouth protruded. See text for discussion of mechanism. Abbreviations: $A_1$, external division of adductor mandibulae muscle; as, ascending process of premaxilla; cpl, cross-palatine ligament; md, mandible; mx, maxilla; mxh, head of maxilla; mx-mdl, maxillary-mandibular ligament; pl, palatine; pmx, premaxilla; pmx-mdl, premaxillary-mandibular ligament.

a complex system of upper jaw ligaments that partially frees the maxilla for new functions.

## CONCLUSIONS

1. There is excellent presumptive evidence for the existence of at least three major adaptive levels in the history of the actinopterygian feeding mechanism. These levels involved a continuum of fundamentally predaceous types extending from the Devonian to the Recent—an interval of nearly 400 million years.
2. The lowest level, represented by the palaeoniscoid fishes, had a limited capacity for adaptive change related to the rigidity of the upper jaw and palate. The basic functional pattern of the actinopterygian feeding mechanism was well established at this level.
3. The modifications that occurred during the palaeoniscoid-holostean transition represented the first radical improvement in the history of the actinopterygian feeding mechanism. They included the freeing of the maxilla from the cheek, an increase in the mass and complexity of the adductor mandibulae muscle, the development of the coronoid process on the mandible, and an increase in torque around the jaw articulation. A change in the position of the interhyal bone permitted more efficient expansion of the orobranchial chamber.
4. The above modifications, which occurred several times independently, permitted considerable adaptive radiation in the feeding mechanism at the holostean level. The basically predaceous pattern persisted, however, in the holosteans ancestral to the teleosts.
5. The holostean–teleostean transition involved no major changes in the predaceous jaw mechanism.
6. The major modifications at the early teleostean level were confined to the palate and jaws. The earliest known predaceous teleosts had already acquired a ball and socket joint between the maxilla and palatine, in contrast to the simple connective tissue joint of the holosteans. In later but still sub-acanthopterygian teleosts, the toothed ramus of the premaxilla was extended posteriorly as a narrow strut below and parallel with the maxilla, excluding the latter from the gape of the mouth and functionally replacing it. These modifications foreshadowed a new mobility for the upper jaw that led to an intensive adaptive radiation of the later teleost feeding mechanism.

7. The origin of the protrusible upper jaw, which characterizes the acanthopterygian level, was the second major refinement in the feeding mechanism. It involved (i) the forward shift of the palatoquadrate and the lower jaw articulation, (ii) secondary simplification of the adductor mandibulae muscles, (iii) differentiation of an adductor component with a tendon to the maxilla, (iv) freeing of the premaxilla from the rostrum, and (v) the development of upper jaw ligaments that, with the maxilla, help to control the forward excursion of the now movable premaxilla.
8. The appearance of a protrusile mechanism incorporating all of the above changes was associated with a hitherto unparalleled increase in adaptive potential of the actinopterygian feeding mechanism. From this basic improvement in jaw mechanics arose the seemingly boundless diversity of feeding specializations for which the acanthopterygian teleosts are so well known.

## ACKNOWLEDGMENTS

We are indebted to Dr. Eigil Nielsen for the opportunity to study the anatomy of the palaeoniscoid and subholostean skull at his laboratory in Copenhagen, to Dr. Donald P. Squires for aid in the analysis of jaw mechanics, and to Dr. C. M. Breder, Jr., for information on teleost feeding habits. Michael Insinna prepared Figures 1 and 4 and aided in the preparation of the other illustrations.

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# JAW MECHANISMS: RHIPIDISTIANS, AMPHIBIANS, REPTILES

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## GENERAL CONSIDERATIONS

Procurement, preparation, and swallowing of foodstuffs are the functions of the jaw mechanisms considered in this study. Other functions also have a direct relationship to jaw morphology—for example, the actions of respiration in fish. These will be introduced only as they pertain directly to one or another specific point in the discussions. In performance of their primary functions, the jaws necessarily have effects upon other systems; and the actions of adjacent systems modify the movement of the jaws. It is not possible to isolate these various actions or to limit or precisely define limits of morphological or functional systems. Jaw mechanisms must be considered from this point of view. We will look at them as a more or less coherent unit with specific functions, but keep in mind that actions and changes external to this system may have strong bearing upon the course of its evolution.

This study is concerned with the rhipidistian ancestors of the tetrapods, the shifts in jaw mechanisms that took place in the development of primitive tetrapods, and the initiation of evolutionary patterns among amphibians and reptiles. It will be emphasized that the principal changes in jaw mechanisms took place after the origin of tetrapods, changes that became possible only after other modifications had occurred.

The actual rhipidistian ancestors of the tetrapods are not known. Jarvik (1942) suggested that there were two sources and that these can be recognized, in general, among the osteolepiform and prorolepiform rhipidistians. Gross (1941) held the opinion that the stegocephalians came from a subgroup of rhipidistians rather different from the ones we know and that the well known rhipidistians are rather distant in structure from the actual ancestors of tetrapods. Nilsson (1944) agreed with this position, emphasizing dental differences between stegocephalians and rhipidistians. The present study is concerned with major modifications; at this level the structure in well known rhipidistians serves reasonably well as a point of departure, even though the relationship to tetrapods is not direct. No decision between single or multiple origins of tetrapods is made. The ichthyostegals are considered to be off the main line of amphibian evolution but, nevertheless, reasonably representative of extremely primitive amphibian morphology. This position is supported by the very primitive amphibian from the Late Carboniferous recently described by Eaton and Stewart (1960).

## RHIPIDISTIAN JAW MECHANICS

*Osteolepis, Eusthenopteron,* and *Megalichthyes* have provided the most substantial information for analysis of rhipidistian jaw mechanics. The studies of Adams (1919), Gross (1941), Jarvik (1942, 1944, 1954), Nilsson 1944), Romer (1937, 1941, 1946) and Watson (1926) have been most used as sources of data for the current discussions and illustrations. The basic morphology is shown in Figs. 1, 2, and 3. The principal morphological features of the jaws, suspensorium, and related structures are as follows: (i) The dentition consists of several rows of small teeth, marginal and medial to the external limit of the jaws, and a few large tusks, above and below, with pits for their reception in the opposite jaws. (ii) The lower jaw is a more or less straight bar. The glenoid articular surface tends to lie well below the line of the tooth row. (iii) The articular surface of the lower jaw is directed postero-dorsally; it is weakly buttressed anteriorly but not posteriorly. (iv) A retroarticular process is present in some genera, as *Megalichthyes,* but this is a specialization, probably not present in ancestors of amphibians. (v) The adductor fossa is long and narrow. All adductor muscula-

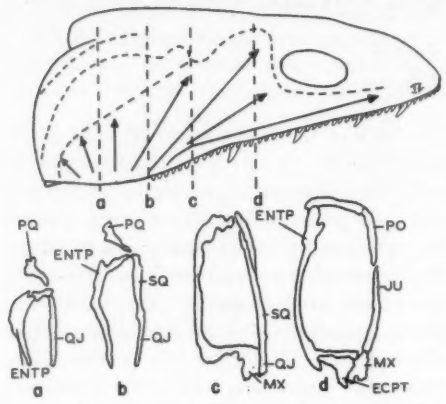


FIG. 1. Diagrammatic reconstruction of the skull of a rhipidistian with cross sections of adductor chamber. Dashed lines indicate limits of palatoquadrate, hyomandibular, and adductor chamber. Arrows show probable extent, fiber direction, and directions of force exerted by adductors. a, b, c, d, sections from posterior to anterior as indicated on reconstruction. ECPT, ectopterygoid; ENTP, entopterygoid; JU, jugal; MX, maxilla; PO, postorbital; PQ, palatoquadrate; QJ, quadratojugal; SQ, squamosal. Drawn from data of Jarvik (1954).

ture appears to have inserted in and on the margins of the adductor fossa. (vi) Depression of the jaw probably was accomplished by the genioglossus and geniohyoideus muscles. A depressor mandibulae of the type found in some amphibians and in reptiles may have been present but there is no clear evidence of it, except possibly in forms with retroarticular processes. Probably a shift toward the development of such a depressor took place within the rhipidistians. (vii) The quadrate portion of the palatoquadrate forms a transverse, rather weakly developed articular region for the lower jaw. It is far posterior, under the opercular region, and lies ventral to the maxillary tooth row. The tooth row slopes dorso-anteriorly, with its posterior end slightly dorsal to the level of the quadrate. (viii) The subtemporal fossa is long and narrow. The adductor chamber in the skull, in which all adductors took origin, is low posteriorly, highest slightly back of the orbit, and apparently extended far forward under the orbit.

From this structural complex the following interpretation of jaw mechanism is derived. Jaw action was limited to simple vertical movements, with little or no lateral or fore-aft shifting upon the jaw articulation. Adduction was performed by a complex of muscles whose general mass can be determined but whose differentiation into components is unknown. Probable fiber directions are those indicated by the arrows in Fig. 1. For the most part, insertions on the lower jaw appear to have been fleshy. There may have been tendinous insertion in the slightly rugose area at the anterior margin of the adductor fossa. Both the outer and inner margins of the adductor fossa are high, and there appears to have been no differentiation in the length of

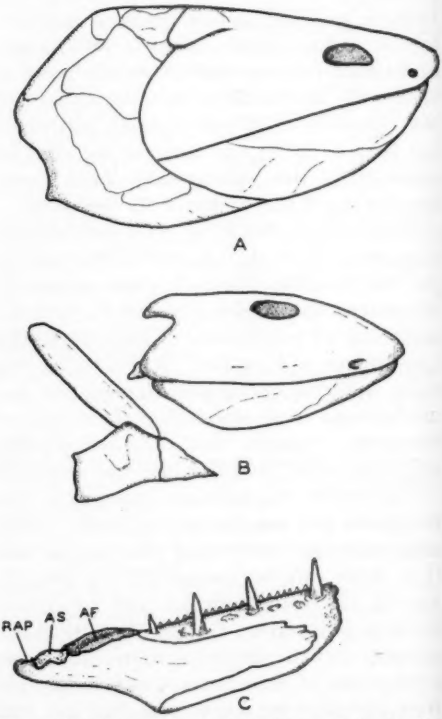


FIG. 2. Sketches of the skulls and jaws of A, *Eusthenopteron;* B, *Ichthyostega,* showing separated shoulder girdle; C, *Megalichthyes.* Not to scale. AF, adductor fossa; AS, articular surface; RAP, retroarticular process. A and B after Jarvik, 1955; C after Watson, 1926.

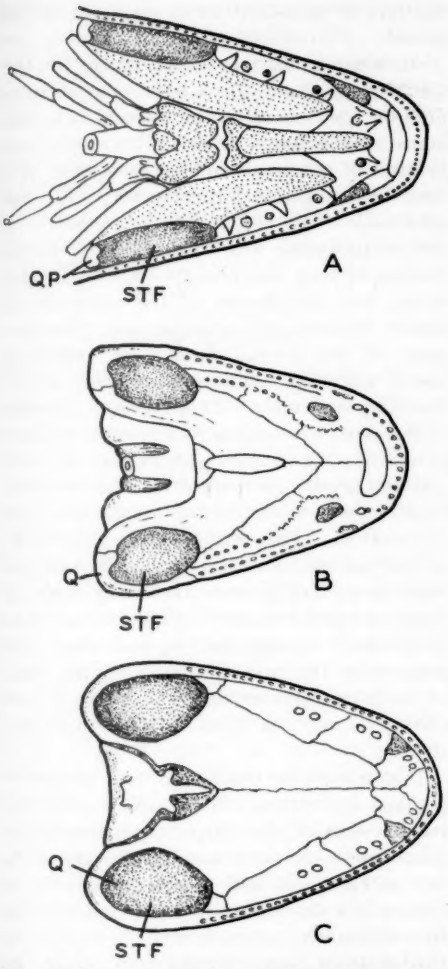


FIG. 3. Palatal views of two amphibians and a rhipidistian. A, *Eusthenopteron;* B, *Ichthyostega;* C, *Loxoma.* Not to scale. Q, quadrate; QP, quadrate process; palatoquadrate; STF, subtemporal fossa. A after Jarvik (1954); B and C after Romer (1947).

muscle fibers that passed to the opposite margins of the fossa.

A main line of force appears to have been developed from the middle of the adductor fossa forward and dorsally to the highest portion of the adductor chamber. In addition, there may have been a rather strong force exerted by the anterior-most part of the adductor complex, a division that probably was somewhat comparable to suborbital muscles in such fish as *Amia* (Allis, 1897). If this portion was significantly developed, as seems probable, it presumably was effective by means of a tendon that passed over the pulley-like anterior end of the subtemporal fossa to insert at the anterior end of the adductor fossa. The main force definitely was antero-dorsal.

The low position of the jaw articulation and related upward slope of the line of occlusion produced a sharply acute angle between the major force vector and the ramus of the jaw in occlusal position. If this interpretation is reasonably correct, it follows that relatively little static force could be exerted when the jaws were at or near closure. When, however, the jaws were at gape so that the forces were exerted approximately normal to the line of the ramus, a potential for strong action existed. At this position, however, static force has little significance. The capacity for exerting strong force, however, could have been expressed kinetically in rapid acceleration of movement of the lower jaw. In a fish-eating predator, such as the rhipidistian, rapid action is undoubtedly a necessity. By virtue of the acceleration, the force exerted near occlusal position was increased by fast actions that were effective until the inertial advantages were overcome. Both piercing by the tusk-like teeth and positioning of prey by the lesser teeth are considered to have been enhanced by this mechanical arrangement.

That there was effective depressor action is a foregone conclusion. Neither the depressors or the nature of related muscles are known. It may be supposed that development of intermandibularis or hyoid muscles was insufficient to provide a thick muscular floor of the mouth or tongue. This is highly speculative, but is to some slight degree supported by the fact that there is no such development in the neotonic stages of modern amphibians in which gill supports are well developed.

### TRANSITIONS TO THE AMPHIBIAN GRADE

The very earliest amphibians, ichthyoste-

gals, are very fish-like in many ways (Säve-Soderbergh, 1936; Jarvik, 1952, 1955). There is, nevertheless, a large gap in the knowledge of transition from the rhipidistians to amphibians, with respect to modifications of both the gill structures and the posterior part of the head and shoulder girdle. The steps can be reconstructed, but the matters of particular concern here, rates and sequences, cannot be understood from presently known materials.

The evidence that early amphibians were largely, perhaps completely, aquatic seems very strong (Romer, 1956b, 1958; Watson, 1926). It also seems certain that these aquatic animals in the adult stage were lung, not gill breathers. The evidence of lack of gill supports is, of course, negative and thus somewhat dangerous. Gill structures, however, are preserved in Permocarboniferous rocks in small larval amphibians and in some in which neotony has become established. It seems probable that had they been present in ichthyostegals, loxomids, and anthracosaurs, they would have left some evidence. If their absence is assumed, it follows that by the time of the earliest amphibians there had been a reorganization of major significance in the areas with which the gills were associated, not only of the hard structures but, more importantly, of the muscles, nerves, and blood vessels. The causes of these sweeping changes, especially in aquatic animals, are as yet obscure. Major modifications also accompanied the changes from "fins" to "limbs," important in the present study as they affect the posterior part of the skull and muscles indirectly related to jaw musculature. If the modifications were adaptive, as seems probable, they must have been adaptive to conditions of aquatic life and their significance should be studied from this point of view. Regardless of the causes and evolutionary significance, the fact that there was major reorganization is important here. Reduction, fragmentation, and possibly loss of the gular plates and branchiostegals accompanied the evident major changes. This undoubtedly had important bearing upon potential mobility and modifications of musculature of the floor of the mouth.

Associated changes took place in the skulls (Figs. 2 and 3). Concurrent with reduction and loss of strong visceral bars and denticulated plates, there was increased ossification of the basicranial region, with closure between the basioccipital and basisphenoid. The highly kinetic structure of the rhipidistian was thus much reduced. Accompanying the change in the basicranium, was the closure of the open dorsal suture between the anterior and posterior parts of the dermal skull roof, reflecting loss of the rhipidistian cranial joint in underlying structures. Proportional changes of the skull were initiated but were realized primarily after development of the amphibian grade. Notochordal aspects of the basicranium were reduced, but far from eliminated. The matter of modifications of dermal elements of the skull roof has been an area of controversy. In general, it does not enter into our considerations. Most of the skull changes did not have direct impact upon the jaw mechanisms, but none of them can be considered as isolated from changes that took place in the jaw structures.

It is concluded that there were profound changes in systems closely allied with jaw mechanisms as the rhipidistian-amphibian transition took place, but the changes in the jaw mechanisms and morphology were in themselves insignificant. It appears that the differences encountered between the rhipidistians and amphibians were no greater than those encountered within the rhipidistians themselves. There does, however, appear to be a directional shift which was not clearly shown in the rhipidistians. The relative position of the jaw articulation was raised. The subtemporal fossa was broadened. The posterior part of the skull was reduced in depth by reduction of the lower part of the posterior wall (Fig. 2). Greater head mobility and greater mobility of the floor of the mouth were developed. These changes all seem to be related to modifications associated with the reduction of the gills and the development of features

of the tetrapod system of locomotion. They were not related to changes in diet.

Although the changes leading to the initial amphibian structure were not per se related to jaw function, their development set the stage for subsequent adaptations. The region was prepared to meet the new challenges posed by the invasion of land, increase in size, and exploitation of other modes of aquatic feeding, beyond simple predation on active animals.

### TETRAPOD SYSTEMS

The jaw mechanics encountered among tetrapods present a somewhat bewildering array of adaptive types. Within the reptiles and amphibians, and to some extent among mammals, however, there appear to be two basic patterns of mechanical action which occur discretely in many cases and in combination in others. One of these we will call the "Kinetic Inertial System" and the other the "Static Pressure System" in order to express their primary characteristics in short phrases for sake of easy reference. These will be abbreviated respectively K-I and S-P hereafter. Being functional, each has an adaptive aspect and thus an evolutionary significance. Where multiple adaptive roles of the jaws and dentitions occur, the two types may occur together and extensive modifications may be encountered. Phylogenetic expressions of the two systems are relatively complex since convergence and parallelism inevitably play important roles. Much of the complexity occurs at adaptive levels beyond those of the primitive reptiles and amphibians and will not be our primary concern.

The two systems may be defined and characterized as follows:

1. K-I system. The effective action of the jaw and teeth depends primarily upon forces developed by rapid motion of the lower jaws relative to the upper. Once motion has been initiated, it is the velocity and mass of the jaws that are effective. The jaws at rest tend to exert little force and in or near occlusal position the forces serve to do little more than hold position. The necessary forces are a force to impart rapid motion to the open lower jaw, a force to hold the jaws in position, and force to open the jaws. The actions are carried out in simple vertical movements.
2. S-P system. The primary action results from pressures exerted by the jaws when nearly closed and initially not in motion. This force produces the stress necessary to overcome resistance of the object to which it is applied, resulting in yielding and subsequent motion. The forces necessary are a strong force developing pressure during initiation of movement, with jaws rather near occlusal positions, forces to induce and resist lateral movement of the lower jaw relative to the upper, force to open the jaw. The actions are more complex than in the case of the K-I system, involving both vertical and lateral movement and, in some instances, fore-aft movement as well.

Given the general structural patterns of the vertebrate jaws, there are clearly a number of ways in which each of the systems could develop. The pattern and function in the rhipidistians was essentially an unprogressive K-I system (Fig. 4). It is possible to visualize a somewhat less specialized stage in which the adductor mass was essentially undifferentiated. Given such a muscle mass and the known types of differentiation among vertebrates, it is evident that emphasis and specialization have been such that three parts of the primary mass are differentiated: an anterior mass, a posterior mass, and one that primitively lies in the vicinity of the anterior margin of the subtemporal fossa. For simplicity and to avoid distracting problems of homologies, these three potential divisions will be termed:

*A-adductors,* the anterior adductors. These are roughly equivalent to the muscles sometimes termed anterior pterygoids.

*P-adductors,* the posterior adductors. These include the "temporal mass" or rough equivalents of the mass from which the external adductors, posterior adductors, and the pseudo-temporals of some reptiles are differentiated.

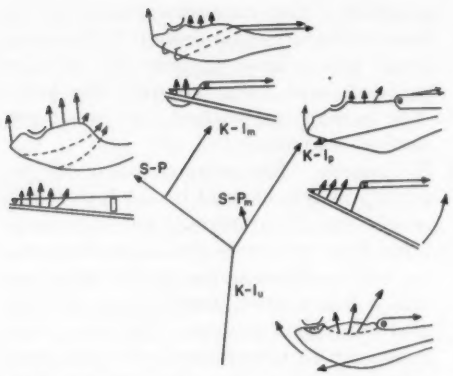


FIG. 4. Diagrams of the patterns of types of mechanical systems of jaws. K-I, the kinetic inertial system; S-P, the static pressure system. Subscripts: u, unspecialized; p, progressive; m, modified. The lines show general course of descent and genetic relationships of types of systems.

*M-adductors,* the middle adductors. This mass takes origin around the anterior margin of the subtemporal fossa, on the dorsal and ventral surfaces of the adjacent palate, and may encroach upon other contiguous structures. It is roughly equivalent to posterior pterygoids of some terminologies.

## THE K-I SYSTEM

### *Development of the system*

This mechanical system was present in rudimentary form in primitive amphibians and presumably was ancestral to all later tetrapod types. In early stages A-adductors were brought into play by a tendon that passed over the pulley-like anterior end of the subtemporal fossa and inserted at the anterior end of the adductor fossa (Figs. 3, 4). This was the case in rhipidistians and primitive amphibians. P-adductors were in large part fleshy, of some importance in adduction, and critical in the holding of jaw position. M-adductors were not developed.

This system became fully expressed among many amphibians. The generalized pattern that arose through this development is shown in Fig. 4. It accompanies evolution of many of the well known trends of amphibian evolution: flattening of the skull, broadening of the skull, enlargement of interpterygoidal vacuities, reduction and loss of kinesis, and progressive chondrification (Romer, 1947). In its greatest simplicity but with a high degree of efficiency, the K-I system may be seen in the morphostages represented by *Eryops* and *Eupelor* shown in Figs. 5 B, C and 6 B, C. These are respectively an intermediate and an end member illustrating the common trend of amphibian evolution toward large, flat, semiaquatic to aquatic animals. The changes witnessed probably were initiated in the loxomids. Some of the changes in mechanics of the jaws are shown in Figs. 4, 5, and 6. The following structural modifications are especially important:

*Musculature:* The P-adductors became very short, massive and with the mean vector directed increasingly medially. The M-adductors failed to develop. The A-adductors enlarged, extended anteriorly, differentiated somewhat into a superficial and deep

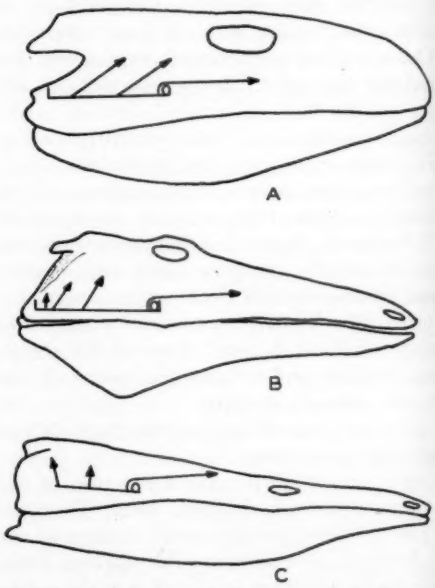


FIG. 5. Lateral views of skulls and jaws of skulls and jaws of A, *Ichthyostega;* B, *Eryops;* C, *Eupelor.* Not to scale. Solid horizontal line in skulls shows length of subtemporal fossa. Arrows show directions and rough relative magnitudes of adductor forces. A after Romer (1947).

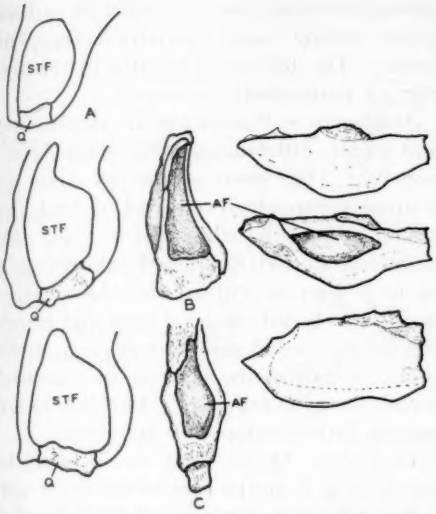


FIG. 6. Diagrams of ventral view of subtemporal fossae, dorsal view adductor fossae, and posterior parts of jaws of A, *Ichthyostega;* B, *Eryops;* C, *Eupelor.* For *Eryops,* both lateral and medial aspects of lower jaw. AF, adductor fossa; Q, quadrate; STF, subtemporal fossa. A after Romer (1947).

part, and extended into a strong tendon that passed over a pulley at the anterior margin of the subtemporal fossa. The origin of these muscles on the skull roof is well shown (Wilson, 1941) in *"Buettneria"* and *Eupelor* (Colbert and Imbrie, 1956). All adductor muscles inserted in or around the adductor fossa, with insertion of the A-adductors by a heavy tendon to the rugose prominence at the anterior end of the fossa (Fig. 6, *see also* Case, 1932). Presumably there was a depressor mandibulae present, but the geniohyoideus and genioglossus probably were supplemental depressors.

*Osteology:* The subtemporal fossa became dual with a large, ovoid posterior part for the P-adductors and a somewhat restricted, slot-like anterior opening for the tendon of the A-adductors (Fig. 6 C). The latter is not to be confused with the superficially similar opening found in S-P systems lateral to the transverse process of the pterygoid. When the lower jaw is in occlusion in forms with the K-I system, the rugose area which carried the tendon rests in the slot, with its dorsal margin about on the level of the upper surface of the palate. The skull becomes increasingly flat; interpterygoidal vacuities become enlarged to serve in part to accommodate the optic structures (levators, retractors, etc.) (Watson, 1951), and in part to accommodate the large A adductors. Kinesis of the palate and brain case is reduced and then completely lost. The suspensorium primitively is far posterior. It tends to move somewhat forward in various types, but does not become braced by adjacent structures such as the stapes or paroccipital. The lower jaws often remain long, with the ramus slender and the adductor fossa about one-fourth to one-fifth of the total length of the jaw. In some end forms, as *Eupelor* (Fig. 6 C), the lower jaw becomes deep and heavy, emphasizing a trend seen in some earlier amphibians. The glenoid articulation remains near the end of the jaw, buttressed in front, open behind. In some genera a retroacticular process is developed. There is generally a small coronoid process, a pseudo-coronoid process in some lines, with the very strong rugosity at the anterior end for the tendon of the A-adductor. The areas of insertion of the adductors remain in and on the immediate margins of the adductor fossa. Emargination of the inner wall of the fossa produced some increase in relative fiber length of muscles to the inner side of the jaw. Teeth accompanying the K-I system are generally sharp and suited to piercing and holding prey.

### *Expressions of the K-I system*

*Eryops* and *Eupelor* as described and illustrated are two concrete examples of the K-I system. It appears to be highly developed in long snouted amphibians such as archegosaurs and trematosaurs. It is likewise well developed in short, broad-headed types in which the rami of the jaws are long, but modified in orientation. In both apsidospondylous and lepospondylous amphibians, K-I is the dominant system. In both lines, of course, there are departures from it. Among labyrinthodonts such terrestrial types as trematopsids and dissorphids show some indications of development

of M-adductors and fairly long P-adductors. It would appear that they were trending slightly in the S-P direction. A-adductors seem to have been little developed in them. In short-jawed lepospondyls, such as *Diplocaulus*, the adductor musculature appears to have been short, heavy, and fleshy. The main action probably was of the S-P type, but with relatively little efficiency or strength.

The K-I system is developed in various reptiles but, for the most part, in a different guise. It has come in most, perhaps all, instances from modification of the S-P system. The most common occurrence is in aquatic forms. In the crocodiles and alligators, as shown diagrammatically in Fig. 4 (*K-Im*) the M-adductors, in the form of pterygoids, are very strong and, holding the jaws in something of a sling, can produce very rapid acceleration, much like that imparted by the A-adductors in the more primitive K-I system (*K-Im* and *p*). Here the P-adductors are weak and serve mainly for positioning and holding. In other aquatic reptiles, the K-I mechanism is developed by elongation of the P-adductors, formation of a coronoid process, and, often, insertion well back toward the fulcrum of the jaw. In such forms (for example snapping turtles, ichthyosaurs, and some mosasaurs) the M-adductors are present and appear to give additional stability and holding power, with the P-adductors converted to other uses.

## THE S-P SYSTEM

### *Development of the system*

This system is one in which pressure is developed when the jaws are not in motion, usually at or near occlusal position. It developed from the unprogressive K-I system of the primitive amphibian through differentiation of M-adductors and emphasis upon P-adductors. It is subject to much greater ramification in evolution, inasmuch as not only vertical but also lateral and antero-posterior movements of the lower jaw are possible. These more diversified actions are, for the most part, developed among mammals, but they occur in various guises among some specialized reptilian stocks. The following features of the system are particularly important.

*Musculature:* P-adductors are emphasized and usually differentiated into several components. They insert on the jaw, often on a strong coronoid process, and on both the lateral and medial surface of the jaw. M-adductors are well developed, taking origin in large part at and around the anterior margin of the subtemporal fossa and inserting on the medial and ventral parts of the posterior half of the jaw, on the external surface in some instances. A-adductors are usually little developed or are absent.

*Osteology:* Skulls tend to increase in depth (Fig. 7) and to remain relatively narrow. The posterior part of the adductor chamber tends to increase in height and depth. Temporal fenestrae tend to develop. The suspensorium moves anteriorly from its primitive posterior position and tends to become strongly supported by subsidiary structures, paroccipital process, stapes, pterygoid, and dermal temporal elements. The palate remains largely intact, without development of large interpterygoidal vacuities. The transverse process of the pterygoid becomes well developed and extended ventrally in a flange. It serves in part for origin of the M-adductors and also buffers the lower jaw against lateral movements in many reptiles. Kinesis, involving the palate and brain case, is present in the primitive stage and persists in many lines. Lower jaws are highly varied in form and show no consistent trends. Increased length relative to body length generally does not occur. A coronoid process tends to develop on the lower jaw at the site of insertion of the major tendon of the P-adductors. The areas in and around the adductor fossa provide for insertion of the P-adductors, but additional insertion occurs on the adjacent faces of the jaws. A strong retroarticular process (reptiles and amphibians) tends to develop, related to the depressor mandibulae and, in some forms to the M-adductors as well.

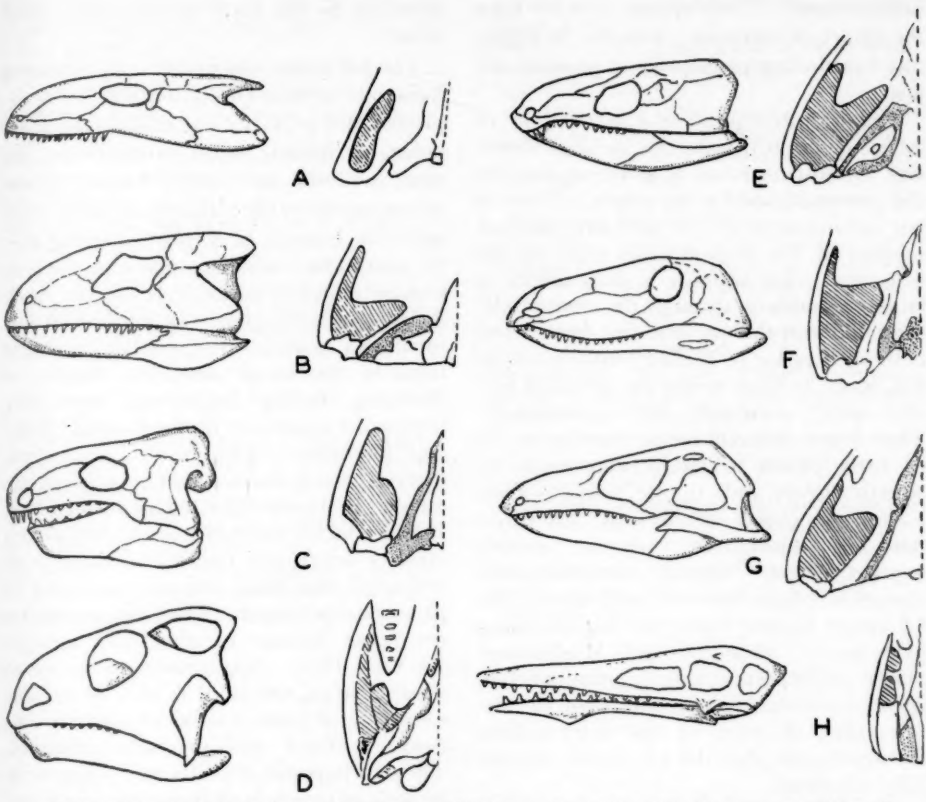


FIG. 7. Lateral views of skulls of various amphibians and reptiles with diagrams of region of subtemporal fossae for each. A, *Paleogyrinus;* B, *Seymouria;* C, *Diadectes;* D, *Trilophosaurus;* E, *Captorhinus;* F, *Ophiacodon;* G, *Youngiodes;* H, *Pterodactylus.* A and B after Romer (1947), rest after Romer (1956a).

*Expressions of the S-P system*

Some of the types of skulls in which the system occurs are shown in Fig. 7. Expression is highly varied. One type of development in which members have a strong otic notch is shown in Fig. 7 A-D. This is seen in the batrachosaurs, which developed directly from anthracosaur ancestors. In neither anthracosaurs or batrachosaurs is the system highly developed. The suspensorium has remained posterior, but has gained some support by expanded otic structures in batrachosaurs such as *Seymouria.* Incipient development seems to be much the same as in dissorophids, perhaps accompanying initiation of terrestrial habitation and feeding. The pterygoid flange is strong in *Seymouria* in contrast to that of dissorophids and trematopsids. In more advanced phases of this type of pattern the suspensorium is swung forward as seen in incipient and full development in procolophonids, in *Diadectes,* turtles, pareiasurs, and *Trilophosaurus.* In all of these animals the otic region tends to be modified to provide strong support to the suspensorium. *Trilophosaurus* appears to represent a separate development of this pattern, a striking example of convergence. In all of these forms, the P-adductors were relatively long, massive, and to some degree

differentiated. They appear to have been the principal adductors, with the M-adductors functioning principally to position the jaws.

*Captorhinus* represents a second type of S-P system, in which the otic notch is absent and the suspensorium is given support by the pterygoid and by the stapes. There is less involvement of the otic structures in support of the suspensorium than in the first type; what support it does render is largely secondary, through the dermal elements. From this pattern, but from a less developed phase of it than *Captorhinus* itself, seems to have arisen the synapsid pattern which eventually led to mammals. This group showed strong tendencies in its early phases to retain adaptations to aquatic habits and, in the case of *Ophiacodon,* to evolve strongly in this direction and initiate an incipient, secondary K-I system. Among non-carnivorous synapsids, edaphosaurians, and caseids, the S-P system became highly developed among pelycosaurs. Although the M-adductors played an important role in primitive synapsid carnivores, it was only with the development of some of the intermediate therapsid types that the S-P system became fully expressed.

The S-P system is encountered among early diapsids, among the younginids, and is carried through into various lines. In many highly specialized forms there are strong modification, and also frequent modifications to K-I systems, particularly in aquatic forms and in some flying types. P-adductors are generally the principal muscles of adduction, and M-adductors serve for positioning and to hold the jaws when at rest. They also serve in some reptiles to provide some of the forces involved in lateral jaw movements. Strong development of kinesis is characteristic of some lepidosaurians, lizards and snakes in particular. When this is carried to very high levels, as in snakes, the principal functions of the S-P system are lost, and there are strong departures from both the primary systems. In snakes, for example, there is a peculiar type of K-I system with the motion provided by the body, not the jaws themselves.

The S-P system was first developed among tetrapods as land habitation was being exploited and probably was initially an adaptation to feeding upon invertebrates, insects, molluscs, and similar forms. It was accompanied by the initiation of other characteristic features of terrestrial tetrapods–in particular, modifications that led to greater mobility of animals on the land. Related to development of jaw action was increasing mobility of the head, modifications of the hyoid structures related to changing feeding habits, and increasing freedom of movement of the shoulder girdle and forelimbs. With initiation of these features (each more demanding upon the muscles in specific roles played by sets, individual muscles, and interrelated complexes) areas of origin and insertion became more restricted and more precisely adjusted to particular functions. Thus the stresses on the bones became localized and stronger (see Case, 1924). Apparently in response to these changes, the skull tended to become a network of lines of stress which were narrowly confined and precisely delimited. The development of skulls which appear to be skeletal networks of these lines are found in various late phases of reptilian evolution. The development of fenestrae and fossae very probably are related to this specificity, with the remaining bone representing the critical lines, and the fenestrae and fossae in a sense negative areas resulting from the loss of bone in non-critical regions.

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# THE FEEDING MECHANISM OF SNAKES AND ITS POSSIBLE EVOLUTION

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## INTRODUCTION

The adaptations of snakes are proverbial and have contributed to making them the most notorious—as well as the most successful—of living reptiles. More than 3000 forms of snakes have become specialized for marine, freshwater aquatic, terrestrial, arboreal, and fossorial modes of life, enabling them to invade all continents (but Antarctica) and many oceans. Most popular attention has traditionally been directed to aspects of the ophidian feeding mechanism, particularly to the use of venom and to the ability to ingest spectacularly large prey.

Recently there has been a revival of interest in snake feeding, as indicated by a series of studies attempting to correlate morphological and behavioral observations (*cf.* Gans, 1952; Dullemeijer, 1956, 1959; Kochva, 1958; Frazzetta, 1959; Albright and Nelson, 1959, 1959a). These studies were based to a considerable extent upon the pioneer analyses of snake head muscles carried out by Haas (1929, 1930, 1930a, 1930b, 1931, 1931a, 1932, 1934, 1952, and 1955) and also by Anthony and Serra (1950, 1951) using a modification of the nomenclature established by Lakjer (1926).

Although these authors proceeded from different viewpoints and used divers nomenclatures for morphological and functional concepts, their studies, and a host of other field and terrarium observations, have yielded a reasonable agreement regarding the major features of snake feeding behavior. Three questions deserve renewed discussion: (i) What was the change that established the selective advantage for a fundamentally different feeding mechanism in snakes? (Or possibly: What change was possible because a different feeding apparatus arose?); (ii) How did such a mechanism evolve?; (iii) What ophidian modifications may be viewed as secondary reflections of the functional and structural shift? The present discussion considers the first two topics simultaneously.

There are several difficulties complicating the analysis, including (i) the remarkable range of adaptive radiation within the superficially simple ophidian feeding mechanism; (ii) considerable disagreement about the purported snake ancestors, or even about the inclusion of certain families within the Ophidia (*cf.* McDowell and Bogert, 1954; Underwood, 1957, 1957a); (iii) the existence of a few supposedly "primitive" genera, modified to such an extent that their poorly understood feeding mechanisms are unique to each; and (iv) the confused homologies of the ophidian cephalic musculature. These real difficulties are here circumvented by keeping the analysis extremely general and by dealing initially with a functional shift from one structural type to another without concern for taxonomic groupings.

There is little argument that snakes originated from some kind of small, tetrapod, carnivorous reptile; certainly none of the recent discussions suggests anything else. The immediately apparent differences between most modern snakes and their hypothetical ancestors are the absence of limbs and the elongation of the body. While it is immaterial whether the change occurred

This essay presents some preliminary ideas on topics that have concerned me for some time. It is an interim statement at best.

I do want to express my appreciation to the many friends who assisted by discussing aspects of these topics and to the authorities of the Buffalo Zoological Gardens (in particular Messrs. W. F. Leumer and C. F. Freiheit) for their courteous cooperation. Special thanks are due to C. M. Bogert, T. Frazzetta, W. T. Neill, and A. S. Rand, who took the trouble to offer detailed comments on the draft manuscript. The responsibility for the final version is that of the author.

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via a fossorial intermediate form (*cf.* Walls, 1942; Bellairs and Underwood, 1951; Underwood, 1957), it is tempting to attribute the shift in the feeding mechanism to the increasing limblessness of the ancestral forms, particularly since the lack of appendages is probably the best known ophidian attribute. However, analysis suggests that elongation of the body rather than the loss of appendages made the functional shift advantageous.

### THE FEEDING METHODS OF LIZARDS

The recent reptiles most similar to the hypothetical snake ancestors are lizards. Observation of their general feeding patterns may furnish a first approximation to the putative ancestral behavior. Extrapolation from such recent behavior, or selection of supposed general trends within it, would seem to involve less danger than would the establishment of an entirely theoretical sequence based upon fossil material alone.

Most tetrapod, carnivorous lizards utilize one of two basic ingestion systems; these systems depend primarily upon the relative size of the prey and thus grade into each other. If the prey is small in comparison to the head it is seized between the jaws and immobilized or killed by crushing and perforation. If the object is large in comparison to the head, so that only a small portion may be grasped at once, it is seized, lifted off the ground, and killed or immobilized by crushing or violent shaking (*cf.* Bogert and del Campo, 1956). Such shaking may including beating the prey against the ground. In some species of *Varanus* the shaking occurs with enough force that it may break the neck of a mammal or bird and rupture the visceral cavity (personal observation).

The biting of arthropod prey is often followed by a series of chewing movements during which the chitin is cut, crushed, or pierced. Hotton (1955) indicates that this, rather than peristaltic motion, furnishes the primary reduction of food items. The piercing action of the teeth should furthermore facilitate the penetration of digestive enzymes. The shift or manipulation of the prey between bites is handled by the flap-like, papillate, muscular tongue. This shifts the prey against the teeth of the upper jaw while the mouth is partially opened and the teeth of the lower jaw are completely disengaged. Similar tongue movements are used for the manipulation of other small prey.

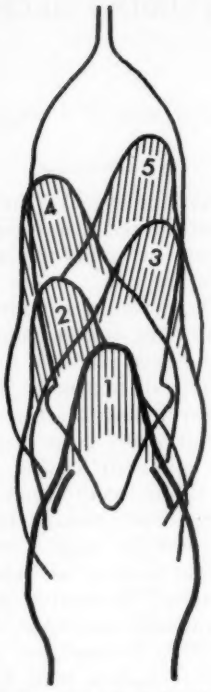


FIG. 1. Schematic view of an inertial feeding sequence. The numbers refer to successive positions of the head. The lateral movement has been exaggerated and the number of steps shortened to clarify the illustration.

Large prey is ingested by an "inertial feeding" method. The jaws release the prey quite suddenly and the entire head shifts forward and laterally, then bites it in a new position (Fig. 1). The inertia of the bulky prey reduces prey shifting. The head continues to shift from side to side in a walking sequence that gradually produces ingestion of the prey. The lateral movements may be irregular with odd-shaped prey, in some forms they may occasionally

be omitted altogether (Frazzetta, *in litt.*). The sequence results in a pushing of the head over the prey rather than drawing the prey into the mouth. The lizard's body maintains its position, while the rapid rostro-lateral shifts of the head (with the teeth free) alternate with a retracting movement (with the teeth engaged). The tongue is not needed for prey manipulation. In some forms, including those that feed exclusively by inertial methods, it has become elongate, deeply-bifid and extensile, serving exclusively for olfactory and possibly tactile sampling of the environment (Bogert and del Campo, 1956; Jollie, 1960).

A certain selective advantage probably accrues to any device promoting rapid disengagement of the upper as well as the lower jaw and it is interesting to speculate on the importance of kinetic movements of the snout in this connection. Some slight posterior curvature of the teeth might have similar utility since the jaw movements (relative to the prey) are primarily rotational, with but slight translational components.

The ingestion sequence involving intermittent shaking tends to produce slight elongation of soft prey. This elongation and the ingestion is often assisted by peristaltic movements of the throat and voluntary neck musculature. The maximum diameter of the prey is limited by the distance between the two quadrato-mandibular articulations, even though authors suggest that cranial kinesis may have some slight effect in increasing this distance.

The key points for the present analysis are that the inertial feeding method increases the maximum size of prey that may be tackled and that the limbs do not assist directly in the feeding sequence . Limbs do not hold the prey, nor maneuver it after it is held between the jaws. Wiping movements of the forelimbs, characteristically employed by many turtles, are almost never seen.[1] The limbs essentially maintain the head and neck above the ground and increase the effectiveness of the inertial feeding sequence by providing a firm base for the rapid shifting movements.

The inertial feeding sequence, repeatedly observed in species of *Varanus, Tupinambis,* and other large lizards, is not restricted to these. Kauffman and Kesling (1960), who analyzed tooth markings left on a large ammonite shell by a platecarpine mosasaur, offer circumstantial evidence for the antiquity of this method. The mosasaur appears to have started with a similar inertial feeding sequence and attempted to extract the soft parts only after the cephalopod proved too large to be swallowed whole. The method is also exhibited by certain snakes such as *Anilius scytale* (personal observation). The semifossorial form ingests elongate lizards and snakes by shifting its entire head from side to side, thus walking the mouth over the prey. An approach to (or departure from) inertial feeding is occasionally used by the large limbless lizard *Ophisaurus apodus.*

### THE LIBERATION OF THE MANDIBULAR TIPS

The foregoing observations make it unlikely that the loss of limbs *per se,* would force the shift to a radically different feeding mechanism. The limbs do facilitate anchoring the body during inertial feeding, yet this anchorage may be achieved by bracing an elongate body against the substratum. Considerable force may be exerted by the body as demonstrated by legless lizards that squeeze prey against the ground, then slowly back up while maintaining the pressure, until the prey may be bitten and ingested.

Recent lizards always show a reduction of limbs (or digits) as a secondary effect of bodily elongation (or of number of presacral vertebrae, *cf.* Sewertzoff, 1931). This suggests that it was this rather than limblessness that established the selective advantage for the new feeding method. Such elongation of the body imposes some unusual burdens upon the head, as it implies a reduced ratio of body diameter to body volume or body mass. If the proportions

[1] Wiping of the sides of the mouth against the ground is very common, particularly after the ingestion of soft or sticky foods. This method may also be used to orient the prey in the mouth.

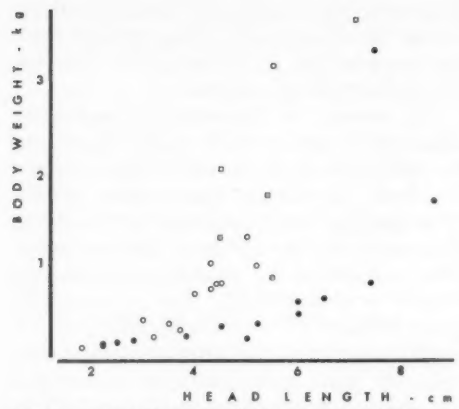


FIG. 2. Scatter diagram of body weight against head length of a random group of colubrid (open circles) and boid (open squares) snakes, and of lizards (solid dots) measured at the Buffalo Zoo. The relatively larger ratio of gape to head length in snakes further accentuates the difference suggested by this diagram.

of the skull (*read:* ratios of skull length to skull width, and skull height to skull width) remain constant, the ratio of body mass to gape length will increase (*cf.* Fig. 2). Compensation for this increase results from: (i) a reduction of metabolic rate; (ii) the ingestion of more, though smaller (as compared to body, *not* to head size), food items; or (iii) a device for the utilization of larger prey. The feeding mechanism of snakes seems to have developed along the third adaptive path, although the first two have also been used in some lines.

Utilization of larger prey involves compensation for its increased diameter. This difficulty may be allayed by biting or tearing off chunks (thus reverting to solution ii) or by increasing the cross-sectional area that can be encircled during ingestion. Possibilities for such increase are limited. The tooth-bearing elements of the upper jaw are kept from spreading by their connection with the brain case and the ocular and olfactory capsules. Some lateral divergence of the quadrato-mandibular articulations is possible, but the obvious and by far the most important expansion occurs at the mandibular symphysis. The utilization of functionally elastic tissues (primarily muscles), here and between the bodies of the mandibles—in fact along the entire ventral surface of the head and neck—will increase the possible vertical dimension of the prey. A further increase in the lateral dimension of the prey becomes possible when the distal tips of the mandibles are able to spread beyond the distance between the quadrato-mandibular articulations. The widest point of the prey will then pass ventral to these joints (*cf.* Fig. 3).

Some motility of the mandibular symphysis is not unusual among lizards. It facilitates fitting the jaws to the prey and reduces the biting forces to be carried by the elements of the skull.

Of the several lines of limbless lizards sharing the reduction of the constricting shoulder girdle to a greater or lesser extent, only the snakes have become truly successful in terms of species abundance, range, and diversity. The closest approach to competition is given by the highly modified amphisbaenids. These efficient burrowers

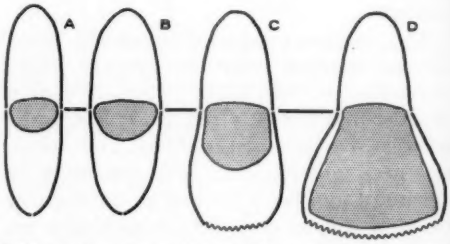


FIG. 3. Four stages in the shift to a mechanism for ingesting larger prey. The mouth is shown at 180° gape, and only the peripheral elements of palate and lower jaw are shown. The line connects the points of quadrato-(pterygo-)mandibular articulation. The length and shape of the elements of the upper and lower jaw have been kept at constant size. The stippled area indicates the approximate cross-sectional area of the largest object that may be passed into the throat. A. Mandibular symphyses firmly fixed. B. Some cranial kinesis, permitting a spread between the two points of quadrato-mandibular articulation. C. Liberation of the mandibular symphysis and stretching of the intermandibular tissues. D. Here the tips of the mandibles are able to spread farther than the distance between the quadrato-mandibular articulations. Note that the widest point of the prey passes ventral to the level of articulation. Possible prey size may be increased further by additional stretching of tissues between the mandibles.

have retained the lizard feeding method and have modified the skull into an excavating tool (Gans, 1960). They have been unable to leave the subterranean habitat and barely 150 forms are found today, in a zone extending only slightly beyond the tropics and subtropics of the Americas and Africa.

The spread of snakes, from what may well have been a similar fossorial zone, into a variety of habitats is undoubtedly correlated with the relatively high level of control and of structural and behavioral modification exhibited by the ophidian locomotor apparatus (*cf.* Gray, 1946; Gray and Lissmann, 1950; Lissmann, 1950; Brain, 1960). Yet the "common denominator" of ophidian locomotion represents little advance over the patterns shown by some lizards.

It is difficult to establish the actual reasons that may have permitted one group and not another to invade a particular adaptive niche. The temporal and geographic factors that may have facilitated earlier spread of one form are unknown, and the presence of the first form may have prevented the success of subsequent invaders, even if these were equally fit from a morphological viewpoint.

In the absence of fossils we are ignorant of the real sequence of adaptations. In my opinion the fundamental modification shared by almost all snakes is the liberation of the mandibular symphysis (and exceptions represent secondary specializations). This was the step that yielded sufficient selective advantage to permit the extreme elongation of the body and the successful invasion of a variety of habitats.

### THE EFFECTS OF MANDIBULAR LIBERATION

The above argument would appear more convincing if it were possible to give reasons for the scarcity of truly functional, as opposed to partially structural, mandibular liberation among lizards. The answer may well relate to the complexity of the muscular and nervous changes required for successful mandibular independence.

As long as the mandibular tips maintain a close (though loose) connection, the muscles need only produce a relatively simple rotation of the mandibular pair about an axis passing through the quadrato-mandibular articulations. Complexity of the muscles in a form with such mandibular connection results from (i) pennation—possibly for increased force, (ii) muscular subdivision into sequentially (?) operated groups, (iii) arrangement of slips to promote kinetic movements, and in some forms (iv) the arrangement of muscles to produce rotational movements about the long axis of each mandible (Kauffman and Kesling, 1960). The third of these factors involves muscle groups that connect to and move the quadrate and pterygopalatal elements rather than the mandible. Rapid opening and closing of the mouth (as required for inertial feeding) can be attained by relatively simple activation of all or part of these muscle groups.[2]

The freeing of the mandibular tips forces a far more complex movement pattern. Spreading of the mandibles involves their rotation about an axis passing along the length of the quadrate, though the motion may be obtained by rotating the quadrate on the supratemporal, or by rotating this bone on the braincase. This motion has to be produced by muscles acting quite close to the rotational axis and against forces exerted on the mandibular tip. The increased rotations and excursions of the ventral tip of the quadrate will involve the pterygoid and through this the palatal shelf and maxillae. Since the mechanical linkages permit extremely complex motions around a series of relatively loose joints, it is necessary to stabilize the system by cradling each mandibular half by a more complex muscle system. Additional stability and simplified coordination may be attained by having mandibular spread initially controlled by the shape of the prey. The increased muscular complexity is at-

[2] The possible subdivision of these muscles into quick-closing (tetanic) and holding (tonic) slips or fiber groupings does not seem to have been studied in reptiles and seems to be beyond the scope of the present analysis.

tained by (i) the shift from short penniform to relatively long, multi-insertion, parallel-fibered muscles and by (ii) the functional shift of muscles. In the second category are some bundles, shifted from support of the skull to control of the quadrate.

The complexity of mechanical linkage systems and of their activating muscles, resulting from mandibular liberation, probably forced a departure from inertial feeding. The rapid and coordinated depression and elevation of two structural elements, separated by a variable distance and connected by elastic tissues at variable tension, may well have required too sophisticated a control system. (This difficulty would not necessarily affect the rapid strike of a snake, since the mandibles are depressed from rest and their tips remain in relatively close juxtaposition.) Mandibular control is much simpler if each side is operated independently. In this context "control" refers to arrangements within the central nervous and neuro-muscular systems.

## THE MECHANICS OF UNILATERAL MANDIBULAR MOVEMENTS

Independent opening and closing of the two mandibles simplifies their control but results in holding action alone. The need for retracting forces, pulling the prey into the throat, has to be satisfied by a modification and shift of the linkages already available for cranial kinesis.

In lizards, the movements of cranial kinesis (Versluys, 1912; Hofer, 1960) consist essentially of a rostrad shift of the distal end of the quadrate and a dorso-rostrad shift of the tooth-bearing elements of the upper jaw (*cf.* Fig. 4). Four steps adapt this system to the new requirements: (i) general loosening of the articulations between the several bones; (ii) simplification of the dorsal attachment between the maxillae and palatines and the nasal capsules (plus their dermal bones), so that the tooth bearing bones may rotate in a horizontal plane; (iii) increased motility of the basipterygoid articulations permitting the pterygoids to follow the latero-rostrad movements of the quadrato-mandibular articulations; and (iv) pointed and slightly recurved teeth, whose shape and manner of insertion will disengage the prey and slide over its surface when the tooth-bearing bones shift forward, and reengage when the tooth-bearing bones retract.

Since the increased complexity of movement is produced mainly by mechanical linkages, these four changes permit the initiation of a major shift in feeding mechanics without a simultaneous and corresponding initial increase in control difficulty. The muscles continue to insert and exert their forces mainly on the quadrates, pterygoids, and the compound bones of the mandible. The latero-rostrad rotation of the distal end of each quadrate automatically produces the forward movements of the tooth-bearing elements. The minimal control difficulty is postulated for the first stage of liberation alone. The complex muscle system of a modern snake requires a complex control, yet this must arise gradually.

The shift to unilateral movements and recurved teeth (Fig. 5) also yields an increased manipulative facility coupled with greater security. The prey's moment of "freedom," midway in the inertial feeding movement, is abolished. One set of tooth rows always retains a firm hold on the prey as the snake swings the toothed elements of each side separately. This implies a greatly increased efficiency giving the unilateral system a distinct selective advantage. This system may even have provided one of the key advantages enabling the snakes's spectacular adaptive radiation. Any such advantage would have come into existence only after the production of a considerable degree of mandibular liberation and unilateral movement. Yet even the beginnings of liberation would increase the engulfing capacity of the specimen, and the discussion has indicated that a limited unilaterality would have been the obvious next step.

It is possible to reverse the entire argument and claim that the loss of limbs was made possible by the feeding mechanism. However, the prey size of lizards seems limited by the capacity of the inertial feeding system rather than the diameter of the cinc-

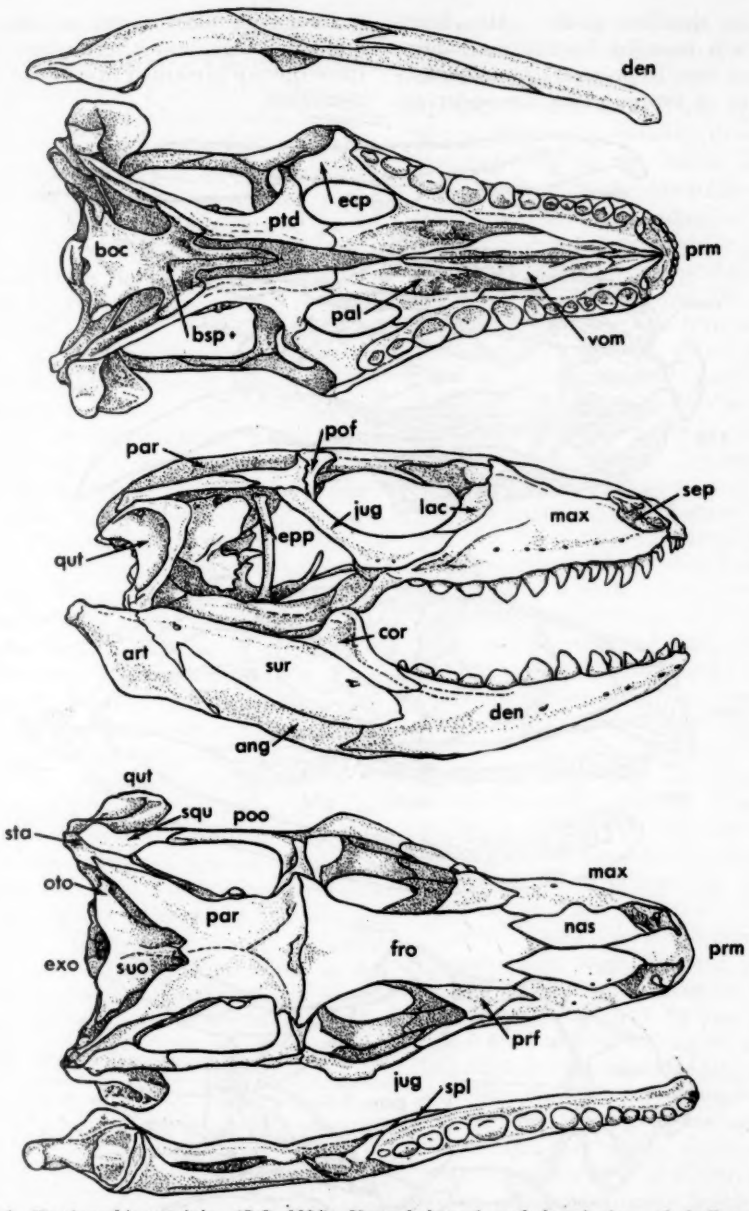


FIG. 4. *Tupinambis teguixin.* (C.G. 1084). Ventral, lateral, and dorsal views of skull and left mandible of omnivorous lizard. Snout-condyle length equals 75 mm. Key to symbols: ang—angular; art—articular; boc—basioccipital; bsp+—basisphenoid plus parasphenoid; col—columella auris (stapes); cor—coronoid; den—dentary; ecp—ectopterygoid; epp—epipterygoid; exo—exoccipital; fro—frontal; jug—jugal; lac—lacrimal; max—maxilla; nas—nasal; oto—opisthotic; pal—palatine; par—parietal; pof—postfrontal; poo—postorbital; prf—prefrontal; prm—premaxilla; ptd—pterygoid; qut—quadrate; sep—septomaxilla; spl—splenial; squ—squamosal; sta—supratemporal; suo—supraoccipital; sur—surangular; sur+—compound bone; vom—vomer. (P. Zuckerman, del.)

ture of the shoulder girdle. Also development of a changed feeding mechanism would then have to proceed simultaneously with a loss of limbs. This argument appears a trifle too complex at this stage of our knowledge, and it seems best to retain the sequence presented first as the basis for discussion.

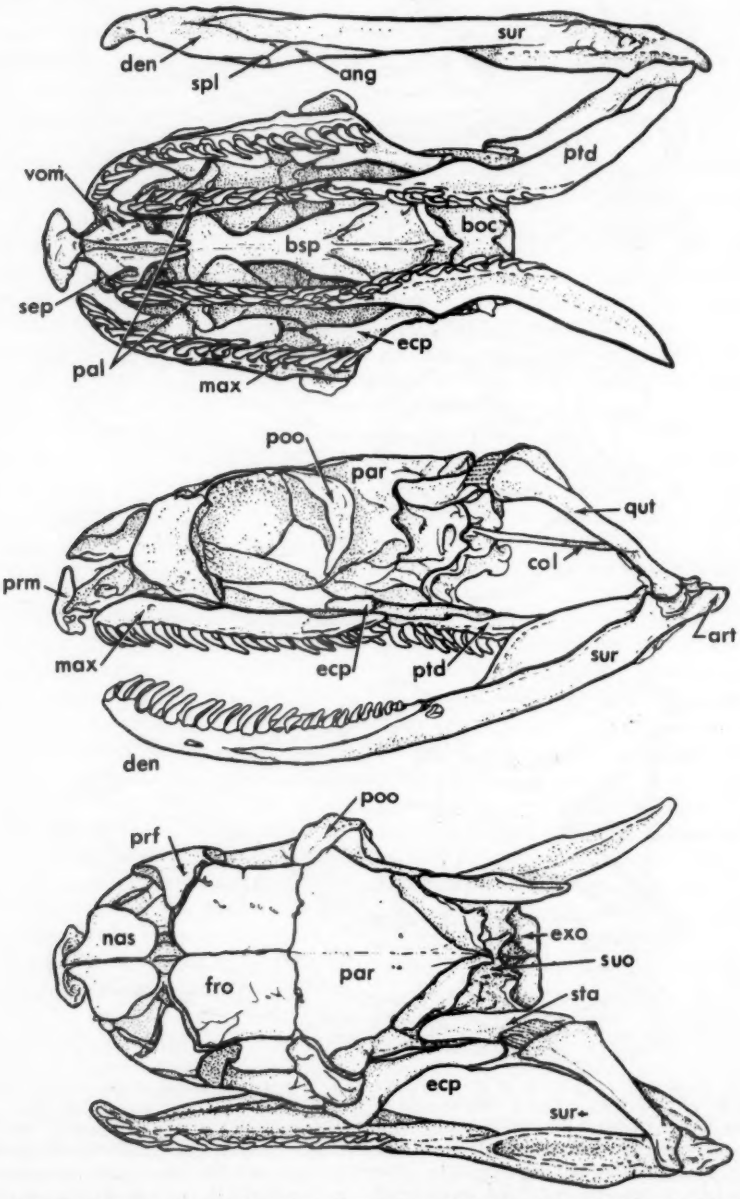


FIG. 5. *Spilotes pullatus*. (C.G. 1075). Ventral, lateral, and dorsal views of skull and left mandible of rat snake. Right mandible and quadrate have been removed. Snout-condyle length equals 28 mm. Symbols as in Fig. 4. (P. Zuckerman, del.)

### THE SECONDARY MODIFICATIONS

The indicated sequence seems plausible in its provision of a gradual sequence of small steps, each of selective value, between the "tetrapod" and snake feeding mechanisms. Secondary modifications without direct functional explanation are not considered in this essay, but a few general modifications will be accorded brief mention. They emphasize the far-reaching effects of a superficially simple change of the ingestion mechanism.

The complete bony investiture of the brain is probably a compensation for the increased looseness of the skull and the complexity of the force pattern. The movement of the "kinetic" joint, from the parieto-frontal to the frontal-nasal-premaxillary zone, was probably forced by this shift.[3] The suspension of the quadrate from the often much elongated supratemporal would seem to be a device for the elongation of the upper and lower jaws and therefore for an increase in the size of available prey. The approximation of the angle of the mouth to the quadrato-mandibular articulation and the elasticity of the skin covering this region permits increased rotation of the mandible about the quadrate. Undulatory movement of the anterior thoracic vertebral column, and of the somatic musculature of this region, pull the prey into the throat, massaging it simultaneously into a more elongate and manageable shape. Many other changes of muscle and ligament arrangement—bone modelling, tooth placement, and articular surface pattern—provide minor improvements of the process. However, each additional detail cited in the course of this functional reconstruction decreases the already tenuous gap between general and special adaptive structures, *i.e.* between those modifications involved in the initial change to a snake habitus and modifications that result from the ophidian adaptive radiation. This is particularly true since we have no knowledge that the "unspecialized" prototype ever existed or that the functional intermediate represents a real animal in the phylogenetic sequence. Studies of variation in a single element, such as those of Anthony (1955), emphasize the difficulty of selecting the directions of specialization.

The modification of feeding behavior also affects several systems and behavioral patterns superficially unconnected with ingestion. These become of importance when prey size is much larger than head size.

Unilateral feeding movements avoid the need for rapid jaw shifts. The prey may be held, rotated, stretched, and otherwise manipulated slowly until ingestion is accomplished. Breathing requires special provision while the snake is feeding. The trachea connects anteriorly to a protrusible glottis that is pushed out beyond the tip of the distended lower jaw. The enormous pulmonary air sacs and tracheal diverticula have also been mentioned as feeding modifications, but their function has not yet been demonstrated.

After the attainment of a certain level of specialization, a snake would theoretically be able to swallow animals too large to be conveniently subdued by the jaws alone. Soft-bodied as well as smaller animals will be killed by suffocation or digestion, as will any other prey that may be kept from climbing out of the esophagous and past the teeth. However, there would seem to be a selective advantage to a preingestion killing of prey which might lacerate or otherwise damage the relatively fragile buccal and esophageal structures. Such advantage increases as the prey size approaches the maximum distension of the jaw linkages.

Constriction of the prey or the use of venom achieves such selective advantage.

[3] This statement expresses my conviction that the primary function of kinesis lies in the mechanical separation of the cranial (*i.e.* brain-containing) and facial (*i.e.* biting) portions of the skull. Continuity of the brain and spinal cord requires that the former be more or less rigidly connected to the cervical vertebrae. Motility of the facial portion may have a variety of uses connected with specializations of the feeding mechanism. In most lizards the brain lies exposed below the frontal bone. The frontal is thus the "logical" element available for the anterior brain enclosure. In assuming this function it has shifted from the facial to the cranial portion in snakes and (independently) in amphisbaenids (Gans, 1960).

Constriction may have started as a squeezing of the prey against the ground (or tunnel wall). Complete encirclement is then a further refinement, and the several methods may attain additional selective advantage by enabling a snake to kill several animals simultaneously and then to eat them in sequence. Alternately, constriction may have been a development secondary to some arboreal specialization (similar to the prehensile tails of various snakes). This topic deserves a detailed analysis of its own.

The use of venom may well be a tertiary specialization; the complex injecting mechanisms certainly are. Yet it may be asked to what extent the use of strong, buccally administered digestive enzymes derives its selective advantage from the system of gulping large prey whole. Introduction of the digestive juices into the circulation of a living animal seems to be an ideal way of promoting their distribution (Zeller, 1948). Digestion may then proceed simultaneously throughout the prey, instead of gradually inward through its skin; and the putrefying action of the prey's viscera may be slowed or modified.

Yet other secondary effects, both metabolic and behavioral, are produced by the mere bulk of the prey (or the inefficiency of large "bites") which requires rest during the digestive periods, possible changes in the chemistry of the digestive enzymes, and a general adaptation to a feast or famine regime. Such effects transgress the proper scope of the present essay.

## SUMMARY AND CONCLUSIONS

The feeding adaptations of snakes are examined from the viewpoint of their functional development.

The initial modification is conceived as a liberation of the mandibular symphysis and a shift from inertial to unilateral feeding. Steps that made this possible are found in the nature of the muscle systems acting on the back of the jaws, and the connection of the tooth-bearing elements in a simple lever pattern that initially kept control problems to a minimum. Important among secondary modifications resulting from the change are the bony enclosure of the brain, the suspension of the quadrates from the extended supratemporal, the protrusible glottis, prey constriction, and the use of venom.

It is suggested that the shift in feeding mechanics was only indirectly related to the loss of limbs and was more directly related to the considerable elongation of the ophidian body. This hypothesis seems slightly more plausible than some others; it is presented in the hope that it will elicit increased consideration of this problem.

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# ORIGIN OF THE MAMMALIAN FEEDING MECHANISM

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The profound and permeating differences between the masticatory apparatus and associated structures in mammals and the corresponding regions in reptiles (and all other vertebrates) have long presented one of the favorite problems of comparative anatomy. The changes that took place in the transition from reptile to mammal are well documented by anatomical, embryological, and more recently by paleontological evidence. The interpretation of some of the homologies is the classical example of comparative anatomy in action. The facts are too well known to warrant more than cursory review: reduction of the mandible from several elements to a single pair of bones, with a completely new articulation between mandible and skull; rebuilding of the middle ear; differentiation of the dentition into fixed regions differing in functon; development of a secondary palate; remodeling of the masticatory musculature; and development of a mobile tongue, fleshy lips, and muscular cheeks. The most radical differences between reptile and mammal are in the rear of the head. The face was much less affected.

The fossil record shows that the transition from reptile to mammal took place gradually over many millions of years. This is an important datum; if we did not have this incontrovertible evidence the temptation to explain so improbable a shift by invoking saltatory evolutionary steps would be almost irresistible. Furthermore—an even more improbable deduction from the data of neozoology—it has become increasingly evident since the suggestion made by Olson (1944) that not one, but several, therapsid stocks exhibit progressive trends leading to the mammalian condition, and that several therapsid lines independently crossed the mammalian threshold.

I am indebted to my colleagues Rainer Zangerl, E. L. DuBrul, and C. A. Reed for extremely helpful discussions during the preparation of this paper.

Thus it is necessary to explain a long-continued, seemingly orthogenetic trend that was generalized throughout an entire stock. Furthermore, this was no minor adaptive trend. There is scarcely a structure in the head that was not affected. Rebuilding of the middle ear and jaw articulation was so drastic that some earlier anatomists scoffed at the idea that these structures could continue to function during the remodeling operation, and therefore refused to believe that the embryological evidence had been correctly interpreted. However improbable the interpretation, the fossil record has proved that it was indeed correct.

The sequence of events in the skull and dentition is reviewed in every textbook of comparative anatomy. The picture comes into sharper focus with each added bit of information, but it is still the same picture that was seen more than a hundred years ago by the anatomists Meckel and Reichert. It is exceedingly unlikely that future discoveries will do more than fill in additional details. The data for the soft parts, on the contrary, have not been properly assembled and reviewed.[1] They can never be as unequivocal as the factual history of the bones and teeth that is supplied by the fossil record; but the changes in the skull can only be described, not understood, if the soft parts are not taken into account, for the skull was only one element—and not necessarily the most important—in a whole functional complex.

It is commonly assumed that this complex syndrome of morphological events was somehow adaptive, but there has been little attempt to understand how and why it happened. The few who have considered

[1] The data have, in fact, been assembled in a broader context and not specifically focused on the present problem, in the several chapters of the Bolk-Göppert *Handbuch* listed in the appended bibliography.

causal factors at all have dismissed the changes in the jaw apparatus as one of the consequential effects of the acquisition of endothermy. Revolutionary changes were taking place throughout the therapsid body, and the triggering factor may indeed have been something remote from the masticatory apparatus. Nevertheless, there must have been primary and consequential events within the masticatory apparatus itself. These can be understood, and the primary factors sorted out from the secondary, only by considering the feeding mechanism from the functional standpoint.

In all living reptiles (except a few secondarily specialized forms of no interest here) the feeding apparatus is *primarily an organ of prehension.* Secondarily it must be able to transfer food from the jaws to the pharynx. Food may be torn apart if it is too large to swallow entire, but it is not masticated. The chunks are swallowed whole. In mammals (again excepting a few secondarily specialized forms) the feeding apparatus is *primarily an organ for the mechanical reduction of food,* only secondarily an organ of prehension. True salivary glands appear for the first time in mammals. In addition to moistening and lubricating the food, saliva adds chemical reduction to mere mechanical reduction. In other words, in mammals the mouth is functionally a part of the digestive system, not merely the entrance to the gut.

Let us examine the differences between the reptilian and mammalian feeding mechanisms from the standpoint of function rather than of comparative anatomy. It is evident that one complex of these differences is directly associated with the treatment of food. The food of reptiles and mammals is essentially the same—often identical—so if structural differences reflect functional differences there must be some really radical difference in the way the food is treated. That difference is simply this: food is mechanically broken down in the mouth of a mammal, whereas in a reptile it is not. The tissues of most plants and animals are tough, and comminuting them requires more than mere pressure; it requires *cutting.* Breaking down the tissues at the beginning of the gut is quite obviously an enormous gain in the efficiency of the digestive process, a gain in the speed and economy with which nutrient materials are made ready for absorption.

The simple conical teeth characteristic of reptiles are designed to puncture. Puncturing an object with pointed projections is a beautifully simple and economical way to enhance the effectiveness of a grasping mechanism. It has been evolved repeatedly during the history of the animal kingdom, and man has utilized this principle repeatedly in designing his machines. But if the object is to be manipulated while it is being held, as living prey must be to bring it into position for swallowing, then the teeth must be temporarily withdrawn at the risk of allowing the prey to escape. The reptiles (among other vertebrates) have solved this problem by making certain parts of the skull—particularly the pterygoid complex—independently movable, so that one part can continue to grasp while another part releases its hold.[2] This kinetic mechanism is operated by a highly differentiated musculature, which may exceed the true jaw-closing musculature in bulk. Kinetism greatly weakens skull architecture, and it disappears when not needed—in herbivorous reptiles, and in aquatic reptiles where the density of water makes the manipulation of prey less hazardous than in air. Among the ancestors of the mammals, kinetism persisted through the pelycosaurs

[2] Various reasons have been suggested for the development of kinetism in the tetrapod skull. Versluys, who studied this problem most extensively, stated in his final word on this subject (1936) that the function of kinetism is "to bring the mouth more to the anterior end of the head than would otherwise be true, perhaps as much as possible into the line of sight of the eyes." This is an astonishingly naive interpretation. Crompton (1935a) believed that "a slightly kinetic skull would absorb the shock when an exceptionally hard bite was taken," which is exactly what a kinetic skull would *not* do. The most plausible suggestion was made by Camp (1930): "kinetism was developed to furnish a powerful swallowing lever . . . the independent mobility of the snout is a release mechanism allowing the prey to be drawn back from the teeth without widely opening the jaws."

and into the early therapsids. It did not disappear completely until mammalian characters were well advanced.

Mechanical reduction of tissues poses three unrelated problems. First is the problem of reducing the tissue to bits. Second is the problem of breathing during mastication. Third is the problem of preventing food particles from entering the respiratory tract. Mammals have solved each of these problems in a characteristic way.

The teeth are the direct effectors in mastication. The gradual change in their function from accessory parts of a grasping mechanism to the primary elements of a machine for cutting and crushing is well documented in the fossil record. It represents a typical adaptive shift, and it is easy to visualize natural selection producing such a straightforward change. A host of other modifications, many of them less obviously adaptive, accompanies this profound shift in the function of the dentition. Changes in those structures that can be fossilized are seen to parallel the changes in the dentition. It is reasonable to assume that this was likewise true of the soft parts. Let us review the more obvious of these changes.

One group of differences is associated with resistance to the deforming and destructive forces developed during mastication. Mastication involves repeated vertical and horizontal movements of the mandible. These movements are carried out against the resistance of the material being chopped up. The magnitude of the forces developed during mastication is unknown, but they certainly greatly exceed the forces developed during prehension. We may confidently assume that the compound reptilian mandible was replaced by a single pair of dentary bones because the compound construction was far less capable of withstanding the forces developed during mastication. The reptiles retained the compound mandible, not because it is architecturally sound, but because it is adequate to the limited demands of prehension. Other reasons for simplification of the mandible have been proposed, but they are less plausible. Parrington (1934), for example, suggested that the dentary expanded to provide for "the proper formation of a coronoid process." There is no obvious reason (other than solidity of construction) why expansion of the already-existing process of the coronoid bone would not have served just as well.

The reptilian skull is poorly designed to resolve strong forces arising in the dental and articular regions. This is obviously true of the kinetic skull, but it is even true of specialized non-kinetic skulls, such as those of turtles or crocodilians. The mammalian skull, on the contrary, shows a high order of architectural integrity. The most important single improvement is the elimination of the quadrate. Once an important link in the mechanism for moving the pterygoid complex, in the non-kinetic skull it seriously weakens the transmission of forces generated in the mandibular articulation. These forces greatly exceed those at any point along the toothrow. The elaborate architectural arrangements in the mammalian skull for resolving forces arising in the mandibular fossa testify to the magnitude of this problem. Thus it appears that one of the most revolutionary differences between reptilian and mammalian skull—the rebuilding of the middle ear—is indirectly attributable to the architectural demands of mastication. It is not yet clear why bones eliminated from the old mandibular articulation were incorporated into the middle ear instead of simply lost, but surely this is not an insoluble problem.

The motor that operates the masticatory mechanism is the jaw musculature. The individual jaw muscles of mammals cannot be homologized with those of any living reptile (Lubosch, 1938). They represent a new and completely different differentiation of the primary muscle anlagen. In part the profound repatterning that took place in the craniomandibular musculature between reptile and mammal can be attributed to the radical changes that took place in the skull, but to a far greater degree it reflects new and simplified mechanical requirements. Gone is the need for motors

to move one part of the upper skull upon another; in mammals the jaw muscles act only to move the jaw in the mandibular articulation. The whole protractor pterygoidei system, which in the Sauropsida moves the maxillary–palatine–pterygoid complex and has no attachment to the mandible, is gone without trace in mammals. This system had stayed with the vertebrates from the fishes on. It is at least suggestive that it atrophies in turtles and crocodilians, which like mammals have completely akinetic skulls. The elaborate internal adductor system of the Sauropsida is likewise attached at one end to the pterygo–palatine complex, but it inserts on the mandible. Thus in kinetic skulls it attaches to movable structures at both ends, so if the mandible is fixed, contraction of this muscle shifts the pterygo–palatine complex. In mammals the whole internal adductor system is reduced to the tiny internal pterygoid muscle. In mammals a premium is placed on speed of jaw closure for seizing, power of occlusion for crushing and cutting, and horizontal movements for cutting and grinding. There is a corresponding division of labor among the muscles: the temporalis for speed, the masseters for power, and the pterygoids for horizontal movements. The pattern of the jaw muscles is singularly uniform throughout the Mammalia, in contrast to the enormous variation in the Sauropsida.

For mastication to be effective, food must constantly be kept in position between the upper and lower toothrows. For saliva to do its job, it must be thoroughly mixed with the food it is to act upon. To accomplish these, the food mass is manipulated with the utmost precision in the mammalian mouth, and there are important new structures to carry out this operation. On the labial side of the toothrow, fleshy lips and cheeks, a completely new development whose musculature was derived from the superficial neck sphincters of reptiles, serve primarily to keep food positioned between the teeth. On the lingual side, the tongue has undergone a change of function. The typical reptilian tongue is a movable part of the mouth floor designed to oppose the pterygopalatine apparatus in the mouth roof when food is maneuvered into position for swallowing. The reptilian tongue is stiffened by the rod-like entoglossal extension of the hyoid bone. In mammals, in contrast, the tongue's job of manipulating food during chewing is at least as important as its role in swallowing. To this end it is a highly mobile organ, largely free of the floor of the mouth, and composed almost entirely of intrinsic muscles. Some anatomists consider the mammalian tongue a wholly new structure—the sublingua representing the remains of the original reptilian tongue. The basket-like reptilian hyoid is reduced in mammals to a mere anchor point for the tongue and larynx, its original function of providing a framework for the pharynx largely taken over by the far more efficient thyroid cartilage.

The intimate association between the entrance to the respiratory tract and the entrance to the gut results in serious respiratory problems when the mouth is used for prolonged processing of food. In typical reptiles the choanae are situated far forward, and the respiratory air stream passes through the mouth cavity on its way to the larynx. This makes respiration impossible when the mouth is full of food and exceedingly dangerous if there are small particles in the mouth, since these would tend to be swept into the trachea along with the air stream. Ingenious arrangements have been evolved for getting around this difficulty. In snakes the larynx and trachea can be protruded from the mouth like a snorkel during feeding, but this is obviously impractical if the snorkel were to be bitten off, as it would be in a chewing animal. A second solution, sealing the respiratory air stream off from the mouth cavity by prolonging the palate backward, was evolved independently several times—in crocodilians, in turtles, in scincid lizards, and again in the therapsid ancestors of the mammals. There is an evident association between loss of kinetism and development of a false palate. In the Therapsida the development of

a secondary palate paralleled loss of kinetism and differentiation of the dentition.

The problem of preventing saliva and food particles from entering the respiratory tract during mastication and swallowing arises partly from the chopped-up condition of the food, but perhaps to an even greater degree from the semi-liquid condition of the food after it has been mixed with saliva. The problem is aggravated by the continuous production of watery secretions from the salivary glands, which would inundate the entrance to the larynx in a reptile. Mammals have an extraordinarily elaborate apparatus to safeguard the entrance to the larynx. The soft palate, the epiglottis, and the palatopharyngeal folds can act together to close off the rear of the mouth cavity, thus isolating the airway completely from contamination by the contents of the mouth, even during mastication. All three of these are peculiarly mammalian structures. Due to an awkward inheritance from pre-mammalian ancestors, food *must* cross the airway during swallowing. The larynx has been radically reconstructed to prevent trouble at this critical moment. The original cricoid and arytenoid cartilages are encased in a new structure, the thyroid cartilage. Another new structure, the epiglottis, protects the entrance to the larynx. Like the prow of a ship deflecting water, the epiglottis deflects food to one side or the other of the laryngeal opening. A whole suite of new laryngeal muscles, split off from surrounding musculature, enormously increases the range and delicacy of laryngeal movement. The thyroid cartilage, which develops from the fourth and fifth visceral arches, has two important functions: to channel stuff on either side of the larynx on its way to the esophagus, and to provide anchorage for many of the new laryngeal muscles.

Thus it appears that the whole cranial revolution that accompanied the origin of mammals, which left its mark on almost every structure in the head, can be attributed to a single primary cause—the mechanical requirements for mastication of food.

It is now generally agreed that the monotremes arose from a different therapsid stock than the therians did. Yet in spite of the extreme specialization of their feeding mechanism, the living monotremes exhibit every feature of the mammalian cranial revolution. Each of these features shows some difference from the therian condition. Differences are often so fundamental that direct common ancestry of individual structures is highly improbable, but *the typical mammalian pattern is always there.* This makes it practically certain that remodeling of the whole masticatory apparatus into the typically mammalian pattern, along with the resulting repercussions throughout the head, took place in the therapsids before the two stocks ancestral to the monotremes and therians separated. Even the origin of such accessory structures as the salivary glands and the thyroid cartilage is then pushed well back into the therapsid stock.

What triggered this remarkable sequence of evolutionary events? The ultimate cause was undoubtedly the selective advantage resulting from increased efficiency in processing food. But we need only look at the birds to verify the fact that, given the cranial morphology of reptiles, multiple solutions to the problem of increasing metabolic efficiency were possible. The fact that the mammalian masticatory syndrome is a mechanically coherent whole does not explain why the therapsid ancestors hit upon the particular solution to the problem that they did. The thing that tipped the scales in favor of the mammalian solution probably lies far back in the early history of the therapsids, or even the still earlier pelycosaurs. It may have been some obscure event in the central nervous system that we can never recover from the fossil record or deduce by comparative anatomy. But whatever the deciding event, a point of no return was quickly reached beyond which the pre-mammalia were committed, beyond which the probability of embarking on a new and different solution was almost nil. From that point on the sequence of events, seen in retrospect, creates an illusion of orthogenesis, but it is only an illusion.

In summary, it appears that the whole mammalian cranial revolution resulted from the mechanical requirements of true mastication. Mastication increases the efficiency with which food is made ready for absorption, and therefore is ultimately related to endothermy. Reduction of food by means of teeth is only one (and not necessarily the best) of several possible solutions to the problem of breaking down food mechanically. By inferring causes from results it should be possible to explain, rather than merely to describe, this remarkable chapter in the evolution of the vertebrates.

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# ACCLIMATION IN MOLLUSCS

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The coastal areas of the tropical seas support a rich and varied invertebrate infauna and epifauna. These animals live at temperatures approaching 40° C. Along the arctic shores the invertebrate epifauna is extremely poor, but the infauna is as rich and diverse as in the tropics (Thorson, 1957). Here, the animals live at temperatures close to and below 0° C. No species has an arctic to tropic distribution, but many have a wide latitudinal distribution and thus may live under extreme temperature conditions. Poikilotherms, as contrasted with homiotherms, do not regulate their body temperature; their body temperature changes with that of the environment. Biological processes are determined by temperature, yet we are presented with the improbable situation that arctic poikilotherms metabolize and grow rapidly in icy waters while tropical poikilotherms often metabolize and grow at a more leisurely pace. The possibility of the existence of compensatory mechanisms in poikilotherms living in colder and warmer seas, was suggested by August Krogh many years ago. In 1916 (p. 101), he wrote, "It would be interesting to compare the respiratory exchange in such cases, because it would appear unlikely from a teleological point of view that it should differ as much as would be ordinarily implied from the temperature difference. One would expect that animals living at a very low temperature should show a relatively high standard metabolism at that temperature compared with others living normally at a high temperature."

We now know that growth rates, respiratory rates, and other rate functions of poikilotherms from different environments do not differ as much as expected from the temperature differences between the environments. Poikilotherms, which are passive conformers to the environmental temperature, show compensatory changes in growth rates and metabolic rates in response to the temperatures encountered in different latitudes (Fig. 1), seasons (Fig. 2), and microgeographic areas (Fig. 3).

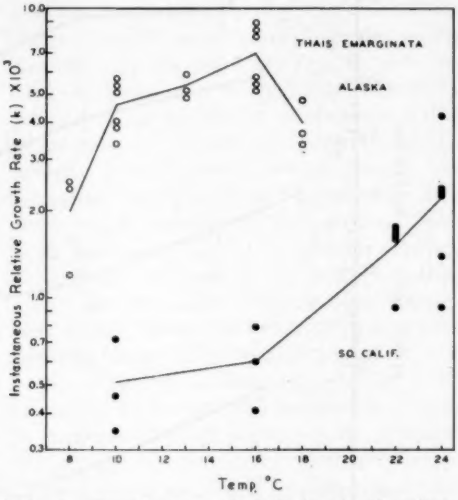


FIG. 1. Growth rates of shell length of veliger larvae of the marine gastropod *Thais emarginata* from Mount Edgecombe, Alaska, and Big Rock, California, as a function of temperature. From Dehnel (1955), by permission of the author and publisher. "Copyright 1955 by the University of Chicago."

Prior to Krogh's work there had been a number of studies purporting to show acclimation[1] to temperature in poikilotherms,

The studies on *Limax flavus* were supported by NSF Grant G-5943 while I was on the staff of Kansas State Teachers College, Emporia, Kansas.

[1] There is no common agreement on the use of the terms "acclimation" and "acclimatization." Some authors make important distinctions between the terms—although not necessarily the same distinctions—others do not. The term "adaptation" is generally accepted as the more inclusive term and thereby acclimation and acclimatization are recognized as a type of adaptation. Since acclimation and acclimatization are arbitrary forms of the same word, I shall refer to any demonstrable compensatory change in an organism as acclimation, to the exclusion of acclimatization, qualified by an adjective appropriate to the influence imposed upon the organism, e.g. "single factor acclimation," "experi-

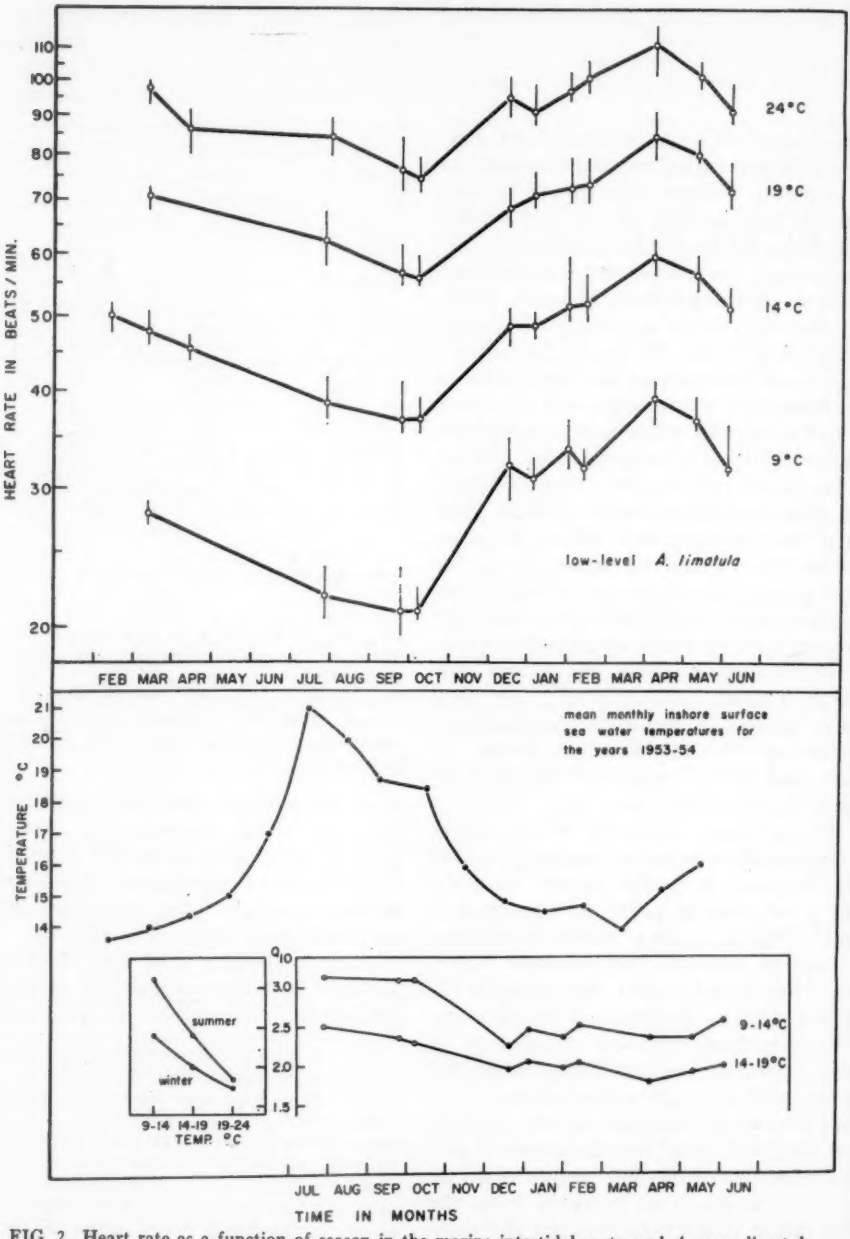


FIG. 2. Heart rate as a function of season in the marine intertidal gastropod *Acmaea limatula*.

but the results of these studies were questionable. The "modern" period began with the work of Fox, Spärck, and Thorson during the 1930's. With the realization of the

mental acclimation," "natural acclimation," "field acclimation," etc. Similarly, qualifying adjectives will be used when the phenotypic or genotypic basis of the adaptation is in question.

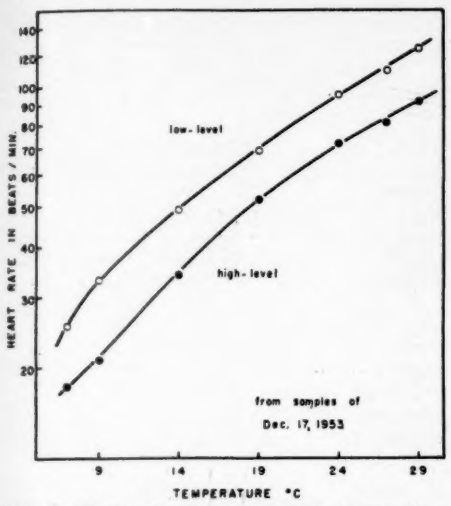


FIG. 3. Heart rate of the gastropod *Acmaea limatula* from different intertidal levels.

significance of acclimation to ecology, distribution, and evolution, there has been a manifest growth of interest in this phenomenon climaxed by the reviews of Bullock, Fry, Precht, and Prosser during the 1950's.

Bullock (1958), has offered four thought-provoking problem areas raised by the phenomenon of acclimation:

1. "The question of the extent and distribution of ecologically important homeostatic mechanisms among groups of organisms by taxonomic category or by habitat category.
2. "The phenotypic versus the genotypic basis for the observed physiological variation among animal populations.
3. "The possible significance of such adaptation for biogeography and the determination of the limits of breeding and of active adult populations.
4. "The question of mechanisms."

Unfortunately we are not much nearer the answers to these questions than we were 20 years ago, in spite of the large numbers of specific examples of acclimation among the molluscs and other poikilotherms.

Bartholomew (1960), clearly stressed the precarious existence of poikilotherms in a changing environment. The overriding problem faced by these animals is that of escape from the severity of environmental change. Reptiles, like molluscs and other poikilotherms, cannot regulate their body temperature by direct physiological activity. But reptiles do regulate, and thereby escape, by behavioral adaptations. Since the portion of the metabolic compensation due to acclimation is moderate compared to the severity of the environmental change, acclimation may play only a minor role as a homeostatic mechanism in reptiles. Molluscs, unlike reptiles, are unable to regulate their body temperature by behavior, thus all of the metabolic compensation permitting escape from the severity of environmental change is due to acclimation.

We must here differentiate between acclimation to the normal temperature range as measured by an activity rate (Precht's "Leistung-adaptation") or "Capacity adaptation") and acclimation to temperature extremes as measured by survival (Precht's "Resistance adaptation"). In those few studies in which both types of acclimation have been examined (*Helix pomatia,* Mews, 1957; *Limax flavus,* Segal, 1959; *Physa gyrina,* Beames, unpublished; *Nodilittorina granularis,* Ohsawa, 1956), both are present in the same organism. In general, warm acclimated animals are relatively heat resistant and cold sensitive whereas cold acclimated animals are relatively cold resistant and heat sensitive. This response is coupled with a shift in the rate-temperature curve so that cold acclimated animals show a higher rate than warm acclimated animals in the range of intermediate temperatures. However, a common physiological basis for both acclimations remains to be demonstrated, and we should continue to regard these as separate processes. Precht (1958) has further suggested that different mechanisms may be involved in acclimation to extremely high and low temperatures.

Since my task is to bring together and interpret the data on acclimation as demonstrated by the molluscs, I feel it will be of value to do so in terms of the aspects of the phenomenon raised by Bullock.

TABLE 1. *Distribution of acclimated response among gastropods*

| Species | Measure | Situation | Authority |
|---|---|---|---|
| Marine | | | |
| *Thais emarginata* | | | |
| *Lacuna carinata* | | | |
| *Crepidula nummaria* | growth (larval) | latitudinal | Dehnel (1955) |
| *Acmaea limatula* | heart beat | intertidal, seasonal, experimental | Segal (1956) |
| *Littorina littorea* | uric acid content of kidney | seasonal | Spitzer (1937) |
| *Nodilittorina granulosa* | extruding response | seasonal | Ohsawa & Tsukuda (1956a) |
| | | geographical | Ohsawa & Tsukuda (1956b) |
| | | experimental | Ohsawa (1956) |
| Fresh water | | | |
| *Ancylus fluviatilis* | | | |
| *Acroluxus lacustris* | $O_2$ consumption | seasonal | Berg (1952) |
| *Physa gyrina* | $O_2$ consumption | hot springs vs. river | Beames (unpublished data, |
| *Limnaea stagnalis* | $O_2$ consumption | experimental | Precht (1939) |
| *Limnaea stagnalis* | excitability of isolated foot | experimental | Benthe (1954) |
| *Limnaea japonica* | growth (larval) | experimental | Imai (1937) |
| Terrestrial | | | |
| *Helix pomatia* | $O_2$ consumption | experimental | Blazka (1955) |
| *Helix pomatia* | $O_2$ consumption | experimental | Gelineo & Kolendic (1953) |
| *Helix pomatia* | $O_2$ consumption, dehydrogenase activity | experimental & seasonal | Kirberger (1953) |
| *Helix pomatia* | Proteolytic activity of stomach juice | experimental | Mews (1957) |
| *Limax maximus* | heart beat ($Q_{10}$) | seasonal | Crozier & Stier (1924, 1926) |
| *Limax flavus* | $O_2$ consumption | experimental | Segal (1959 & unpub. data) |

TABLE 2. *Distribution of acclimated responses among pelecypods*

| Species | Measure | Situation | Authority |
|---|---|---|---|
| Marine | | | |
| *Mytilus californianus* | pumping rate | geographical | Rao (1953) |
| *Mytilus californianus* | pumping rate | intertidal | Segal et al. (1953) |
| *Mytilus californianus* | heart beat | geographical | Pickens (unpub. data) |
| *Mytilus californianus* | shell growth | geographical & intertidal | Dehnel (1956) |
| *Mytilus edulis* | $O_2$ consumption | seasonal | Bruce (1926) |
| *Mytilus edulis* | $O_2$ consumption (gill tissue) | seasonal | Schlieper (1957) |
| *Mytilus edulis* | $O_2$ consumption | geographical | Spärck (1936) |
| *Ostrea gigas* | ciliary activity | seasonal | Usuki & Sadaaki (1954) |
| *Venus mercenaria* | $O_2$ consumption (tissues) | seasonal & geographical | Hopkins (1946) |
| *Siliqua patula* | growth | geographical | Weymouth et al. (1931) |
| *Mya arenaria* | growth | geographical | Newcombe (1936) |
| *Tivela stultorum* | growth | geographical | Coe & Fitch (1950) |

Tables 1, 2, and 3 show the distribution of the acclimated response among the Mollusca. Acclimation has been demonstrated in gastropods, pelecypods, and one species of amphineuran. No studies on cephalopods and scaphopods have been reported. Species have been studied from the three major habitats—marine, fresh water, and terrestrial. Oxygen consumption, heart rate, growth rate, and enzyme activity have been used to demonstrate acclimation in response to laboratory and naturally occurring differences in temperature.

Some molluscs do not show acclimation when subjected to specific tests. However, the absence of the acclimated response for a given measure does not preclude its presence for another measure or for different conditions. Seasonal acclimation is particularly difficult to demonstrate. Two factors may markedly influence the metabolic rate of the organism and thus obscure the com-

TABLE 3. *Resistance acclimation—Shifting of tolerance levels*

| Species | Temperature level | Authority |
|---|---|---|
| Gastropods | | |
| *Physa gyrina* | high | Beames (unpublished data) |
| *Helix pomatia* | high (proteolytic activity of stomach juice) | Mews (1957) |
| *Limax flavus* | low | Segal (unpublished data) |
| *Nodilittorina granularis* | high (seasonal) | Ohsawa & Tsukuda (1956a) |
| *Nodilittorina granularis* | high and low (experimental) | Ohsawa (1956) |
| *Littorina littorea* | | |
| *Littorina rudis* | | |
| *Littorina palliata* | high (intertidal) | Gowanloch & Hayes (1926) |
| *Patella vulgata* | | |
| *Patella depressa* | | |
| *Patella athletica* | high (seasonal) | Evans (1948) |
| Pelecypods | | |
| *Mytilus edulis* | high (gill tissue) | Schlieper & Kowalski (1956) |
| *Lasaea rubra* | high (intertidal) | Morton et al. (1957) |
| Amphineura | | |
| *Clavarizona hirtosa* | high (intertidal) | Kenny (1958) |

pensation to the temperature change. In many species the metabolic rate becomes very high during the breeding season and the breeding season not infrequently coincides with the warmer environmental temperatures. Many other species undergo a winter rest phase with a decline in the metabolic rate.

We cannot correlate the ability to acclimate with habitat in the molluscs. Among gastropods there are good examples of acclimation from marine, fresh water, and terrestrial habitats. Not only do molluscs from all three habitats show acclimation, they show the same "amount" of acclimation. This means that we cannot accept the belief (Scholander et al., 1953; Fry, 1958) that adaptations of terrestrial, and possibly fresh water, poikilotherms are largely related to the resistance to the annual temperature minimum and maximum. *Helix, Limax, Limnaea, Physa, Ancylus,* and *Acroluxus* show acclimation of various activity rates which do not differ in their order of magnitude from that shown by various marine molluscs.

Can we correlate the ability to acclimate with distribution within a given habitat? That is, can we show that species with a wide distribution relative to environmental temperature (eurythermal) acclimate more than species with a narrow distribution (stenothermal)? The converse—are they eurythermal because they can acclimate—may well be the proper question here. Schlieper (1959) maintains that species in cold deep waters, such as *Pinna pectinata* and *Avicula hirundo,* display little or no thermal or osmotic compensation, whereas, species living in surface waters with varying temperatures and salinities do show such acclimation. Schlieper and others have fostered the notion that acclimation is limited to eurythermal forms. It is true our most important knowledge of acclimation has come from intraspecific studies of eurythermal species (Tables 1, 2, and 3). But, some stenothermal species have shown seasonal, and laboratory acclimation. It is unfortunate that critical tests of Schlieper's hypothesis have not been carried out. But the work of Orton (1923), Spärck (1936), Takatsuki (1929), Thorson (1936, 1950), and Wingfield (1939) has shown that species with a northerly distribution have a faster metabolic rate and heart rate than southerly distributed species of the same genus (e.g. *Mytilus,* from arctic and boreal waters, and *Ostrea,* from tropical and northern waters). Developmental rates may also show interspecific acclimation since closely related northern and tropical prosobranch

gastropod larvae seem to spend a similar time in the plankton (about 3 months). There is good evidence (Bullock, 1955 and Thorson, 1956) that the replacing species of many level-bottom genera of molluscs and other poikilotherms are totally or nearly "adapted" to the temperatures under which they live. Scholander et al. (1953), in an extensive study of tropical and arctic marine poikilotherms, conclude that there is considerable, although incomplete, metabolic adaptation in the arctic species relative to the tropical species.

Such interspecific comparisons are difficult to evaluate. Homeostasis of rates in replacing species of a genus may be an expression of a genetically fixed adaptation to habitat temperature. Thus each species lives only where it can, and, as Thorson (1957, p. 205) says, ". . . this adaptation allows each genus to be represented in a sea area by just that species which is able to carry out the normal functions of the genus concerned at the prevailing conditions." However, homeostasis of rates may be evidence of a perfectly good ability to acclimate even though each species has relatively narrow infaunal limits.

This leads directly into the second problem area—the phenotypic versus the genotypic basis for the interpopulation differences in physiological rates and thermal limits. The question has meaning only among natural populations which have an intertidal or geographical distribution coinciding with temperature differences and thus with differences in rate functions. In laboratory and seasonal acclimation, the question has no meaning; the compensatory response is obviously phenotypic. There are tests available for disclosing the nature of differences between natural populations—survival polygons, measurements of the $Q_{10}$ or Arrhenius $\mu$ for various activities, and, what is particularly revealing, reciprocal transplantation and subsequent measurement of one or more activity rates (see Prosser, 1957, for a thorough discussion of this point). The majority of the latitudinally separated populations of species which show physiological differences have not been subjected

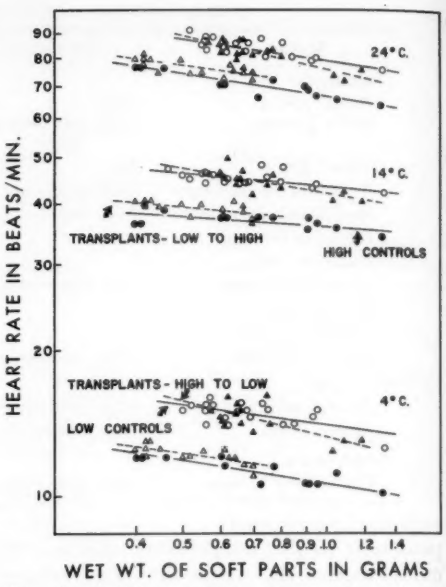


FIG. 4. Relation between heart rate and wet weight of soft parts of reciprocal transplants and controls of the gastropod *Acmaea limatula* 29 days after transplantation.

to these tests. I have used the reciprocal transplantation technique to demonstrate a phenotypic temperature effect on heart rate of intertidal populations of *Acmaea limatula* (Fig. 4 and Segal, 1956). We will have no idea of the extent of racial differences between geographically separated populations of a species unless comparable latitudinal transplantation experiments are carried out.

Loosanoff and Nomejko (1951) reciprocally transplanted adult *Crassostrea virginica* between Virginia and Long Island. The Virginia oysters failed to spawn in two years whereas the Long Island oysters spawned earlier than they normally did in northern waters. Since the Virginia oysters normally spawn at 25° C and the temperature of Long Island Sound failed to reach 25° C during the two years of the study, the authors concluded that the northern and southern populations of *C. virginica* represent physiological temperature races. Korringa (1957) has compiled data to show that there are at least 3 different populations of

the European flat oyster, *Ostrea edulis,* which differ not only in the temperature at which release of the larvae can be observed (17°, 20°, and 25° C), but also in the temperature required for maturation of the gametes. Korringa concluded that there are several physiologically different races of *O. edulis* breeding at different temperatures. Commercial oystermen have conducted large scale transplantation of *O. edulis,* but spat have never been transplanted. A better foundation for racial differences within the populations of each species would certainly be established if both younger and older oysters had been transplanted. Stauber (1950) has suggested that populations of the oyster drill, *Urosalpinx cinerea,* from Delaware and from Virginia, should be considered as physiological species. The geographically separated populations have different temperature thresholds for moving, drilling, and ovipositing. However, controlled transplantation experiments have not been carried out on *Urosalpinx.* Forbes and Crampton (1942) appear to have uncovered physiological races of the fresh water snail *Lymnaea palustris* from Connecticut and from New York. These differ in growth rate, fertility, and longevity under similar laboratory conditions. These differences persisted for three successive generations. To my knowledge, this study has not been followed up with acclimation tests of the natural populations.

To illustrate the third problem area—the limits of the acclimation and the geographical boundaries of species—I will use my own work on the pulmonate gastropod *Limax flavus.* I have not found any previous study on acclimation limits in molluscs. Fig. 5 shows the rate-temperature curves of oxygen consumption for animals maintained at 2°, 5°, 10°, 20°, and 30° C and measured at a series of temperatures from –4° to 30° C. *Limax flavus* compensates for the difference in temperature so that, in general, animals acclimated to the cold have a higher rate of oxygen consumption than animals acclimated to the warm when both are measured at the same temperature. This is

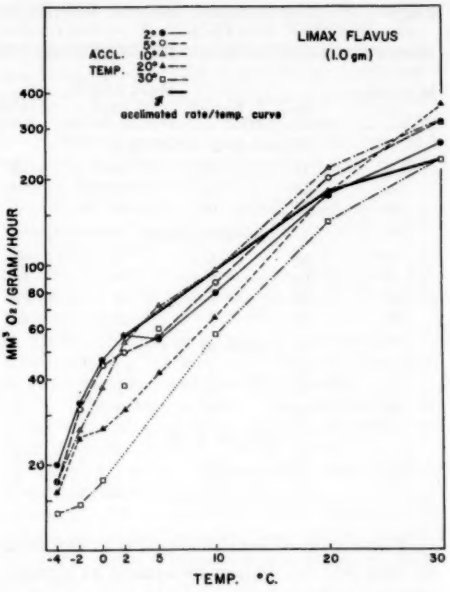


FIG. 5. Relation between oxygen consumption and temperature in the slug *Limax flavus* after acclimation to a series of temperatures.

the classic picture of acclimation of a rate function to temperature and need not concern us further. If one connects the points representing the oxygen consumption of animals acclimated to and measured at the same temperatures (2°, 5°, 10°, 20°, and 30° C), one has an acclimated rate-temperature curve. These animals were given sufficient time to become completely acclimated at each temperature: thus the curve, supposedly, is the more natural curve and shows what the animals do at their normal habitat temperatures. Of course, this is only true if we equate the constant temperature of the laboratory with the integration of the fluctuating temperatures the animals experience in the field. Bullock (1955, 1958) suggests that the acclimated rate-temperature curve will show (1) a slope indicating the degree of sensitivity to temperature, (2) a length indicating temperature range, and (3) a shape at the ends indicating the sharpness of geographic or ecologic limits. The last point is of particular importance here.

TABLE 4. *Survival of* Limax flavus *at low temperatures*

| Acclimation temp, °C | No. | Dead within 3 days: Frozen | Survived |
|---|---|---|---|
| *Twenty-four hr at −4°C* | | | |
| 5 | 10 | 3 | 1 | 6 |
| 10 | 10 | 1 | 0 | 9 |
| 20 | 11 | 0 | 10 | 1 |
| *Five hr at −5°C* | | | |
| 2 | 32 | 6 | 5 | 21 |
| 5 | 25 | 2 | 7 | 16 |
| 10 | 22 | 1 | 0 | 21 |
| 20 | 30 | 4 | 2 | 24 |
| *Five hr at −5.5°C* | | | |
| 2 | 22 | 6 | 15 | 1 |
| 5 | 11 | 6 | 1 | 4 |
| 10 | 11 | 3 | 3 | 5 |
| *Five hr at −6°C* | | | |
| 2 | 21 | 14 | 6 | 1 |
| 10 | 11 | 10 | 1 | 0 |

The limits of acclimation in *L. flavus* are 2° and 30° C. When measured at temperatures in the physiological range, animals kept at 2° and 5° C consume more oxygen than animals kept at 20° and 30° C. But animals kept at 10° C show the highest rate of all. The lower end of the acclimated rate-temperature curve is therefore the curve for the response of the best adapted animals (10° C). It appears as though *L. flavus* has an optimal acclimation temperature; the animals acclimated to the extremes are not necessarily the ones that do best at those temperatures. The limits of acclimation in *L. flavus* do not seem to determine the sharpness of transition between physiological and lethal temperatures and therefore the sharpness of geographic or ecologic limits. This point can also be illustrated by survival of *L. flavus* at low temperatures (Table 4). Animals acclimated to 2° and 5° C appear less well equipped to tolerate low temperatures than animals acclimated to 10° C.

The last problem area deals with the mechanism of acclimation. Acclimation to temperature has been demonstrated at the many levels of biological organization—whole animal, organ, tissue and cell. The data is conflicting in that animals which show metabolic adaptation at the whole animal or organ level, may or may not show adaptation of tissues and enzyme systems. In some animals the nervous and endocrine systems appear to play a definite role but the general belief is that acclimation occurs at the cellular level. Demonstration of acclimation of isolated tissues and of certain enzyme systems in gastropods and pelecypods (see Hopkins, 1946; Kirberger, 1953; Mews, 1957; Schlieper, 1957; and Schlieper and Kowalski, 1956) has contributed to this belief. This information is all we have on the mechanism of acclimation in molluscs. General discussions of the possible mechanisms of temperature acclimation in poikilotherms may be found in Bullock (1955) and Precht (1958). Some of the most challenging problems in the study of acclimation center about our understanding of the nature and site of the mechanisms involved. Increasingly, the approaches at the cellular level are more quantitative. We may soon be able to determine whether acclimation depends on shifts in enzyme pathways, synthesis of more enzyme, or changes in the protein structure of enzymes. We may discover that acclimation depends on other quantitative changes at higher levels of organization.

## SUMMARY

Molluscs and other poikilotherms do not regulate their body temperature yet show varying degrees of homeostasis of rate functions and shifts in tolerance levels in response to the temperatures encountered over the seasons, across the latitudes, in different parts of a microgeographic range, and under experimental manipulation in the laboratory. This compensatory response or acclimation to temperature has been demonstrated in various gastropods and pelecypods and one species of amphineuran from marine, fresh water, and terrestrial habitats; the order of magnitude is similar among all the molluscs. Most of our knowledge of acclimation to temperature in molluscs has come from studies of eurythermal species, but we do not have sufficient data to permit us to say that stenothermal species do not or are less able to acclimate than eurythermal

species. Among eurythermal species, the question has been raised as to whether the homeostatic compensatory adaptations of widely separated populations has a phenotypic or genotypic basis. Out of the welter of cases, only a few appear to have a genetic basis, thus separating the populations as physiological races.

We have little information on the limits to which an animal can be acclimated with respect to temperature. From studies of mine on the pulmonate *Limax flavus* it appears that the limit to which an animal may be acclimated is not necessarily the temperature at which it will show its greatest ability to acclimate. *Limax* shows an optimal acclimation temperature both in metabolic compensation and in survival at low temperatures.

In some animals there is much evidence that nervous and hormonal factors play a decided role in temperature acclimation. But, the demonstration of acclimation of isolated tissues and of certain enzyme systems points to the cell as the site of the mechanism. The challenging problem, today, is the nature of the mechanism.

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# THE COMPARATIVE PHYSIOLOGY OF DIGESTION IN MOLLUSCS

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The comparative physiology of digestion, even when limited to a single phylum like the molluscs, covers a tremendous area. A full review would have to include a discussion of the mechanics of ingestion, those of moving the ingested food through the alimentary tract, the trituration of food substances by mechanical and chemical means to (molecular) sizes which can pass the lining epithelium of the gut (digestion proper), the crossing of the intestinal wall by these digestion products (resorption), and finally the processes of defecation. To this subject belong also processes of secretion (extrusion and restitution), the effects of climate, diet, age, etc., on the digestive processes. Since a review of all these aspects and processes is obviously impossible in a short paper, only a comparatively few problems pertaining to digestion will be presented here.

## ENZYMES AND SECRETION

The molluscs have a surprising enzyme equipment. Indeed, there seems to be no other group in the animal kingdom with such an array of digestive enzymes, particularly the carbohydrases. Apart from the common carbohydrases like amylase, maltase, saccharase, etc., we meet those like lichenase, inulase, xylase, cellulases, hemicellulases, chitinase, etc. These "special" carbohydrases are not limited to groups and species, characterized by highly specialized diets, but are found in Amphineura, Gastropoda and Lamellibranchia. In the Cephalopoda they seem to be lacking (Mansour-Bek, 1954). Recent investigations by Florkin and co-workers have cast some doubt on the validity of the concept that all these special carbohydrases are produced by the animals themselves. Florkin and Lozet (1949) showed that in *Helix pomatia* cellulase is produced by (symbiotic?) bacteria. Jeuniaux (1950, 1954) investigated the origin of chitinase and also found that bacteria, not the snails (*Achatina fulica, Helix nemoralis, H. pomatia, H. aspersa, Limax cinereoniger, Agriolimax agrestris, Succina putris*) produce this enzyme. Jeuniaux (1954) also found that the concentration of bacteria in the snails increases during long periods of rest (aestivation, hibernation) or during periods of starvation. Parallel to this increase in bacterial number an increase in chitinase was apparent (Table 1). Disregarding endogenous rhythms, the secretion of enzymes is usually at a minimum during starvation, aestivation, and hibernation, so that the increase in chitinase activity must be caused by the bacteria. It may be pointed out here that the chitinase activity does not seem to depend on the diet: fungus- and toadstool-eating limicides do not have an exceptionally high chitinase content. Future investigations on species believed to produce such special enzymes themselves will be necessary to show whether this claim is correct or not. If not, we must accept the fact that the array of digestive enzymes produced by molluscs is no more extensive than that of other animals.

Proteolytic enzymes such as proteases, poly- and dipeptidases are found everywhere, as could be expected. Cathepsin has been detected in *Ostrea, Mytilus, Mya* (Rosén, 1949, cited from von Buddenbrock, 1956), *Helix* (Rosén, 1934, 1937, 1941), *Achatina* (Prosser and van Weel, 1958), *Octopus* (Sawano, 1935). Tryptic proteases

TABLE 1. *(after Jeuniaux)*

| Snails | Chitinase units | |
|---|---|---|
| | Stomach | Intestine |
| nourished | 1.0 | 0.25 |
| nourished | 1.7 | 0.40 |
| aestivation | 2.6 | 0.90 |
| hibernation | 2.6 | 0.90 |
| starvation | 3.4 | 1.80 (midgut)<br>0.60 (hind gut) |

are found in cephalopods (Sawano, 1935, Romijn, 1935), *Patella* (Graham, 1934), *Aeolidea* (Graham, 1937/1938), *Ostrea* (Yonge, 1926, Sawano, 1928), *Ensis* (Graham, 1931), *Mya* (Mann, 1949), *Achatina* (van Weel, 1959).

Esterases seem to be common everywhere. Lipases, when present, are as a rule weak.

Surprisingly enough there does not seem to be a big difference in enzyme equipment between the lower groups and those higher on the evolutionary ladder, although data on a group like the Amphineura are admittedly meager. Nor does there seem to exist an appreciable difference between species having different diets. Food specialization, therefore, does not seem to exclude certain enzymes in molluscs, as is the case in highly specialized parasites, such as certain blood parasites.

What about the specialization of enzyme producing glands? In all molluscs, except for the lamellibranchs, salivary glands are present. All molluscs have a usually conspicuous midgut gland (so-called liver or hepato-pancreas) or digestive diverticula. Even the Solenogastres, although lacking a typical gland, do have one or more coeca which may have the same function as the midgut gland. Buccal glands and unicellular mucous glands can be found in most molluscs in the epithelium and underlying tissues of the mouth, and the intestinal tract. Such a morphological separation, concentration, and specialization of glands and gland types suggests a physiological specialization in secretion also. It is well known that secretions with an extremely low pH do occur in certain species: *Dolium galea* produces a strongly acid saliva, supposedly $H_2SO_4$, to a strength of 4%! However, Kühnelt (1929) believes this to be an organic, not a mineral acid (von Buddenbrock, 1956): he could find no HCl, $H_2SO_4$, or $HNO_3$ in the saliva collected from fresh animals. Future investigations should be undertaken to determine what kind of acid is secreted. The toxin-producing salivary glands of cephalopods and the toxin gland of *Conus* are other examples of specialized glands, producing a secretion not found elsewhere in the digestive system.

Although the salivary glands produce chiefly carbohydrases, proteases are not rare in carnivorous molluscs (*Murex, Buccinum, Sepia, Octopus*). They have even been found in certain herbivorous ones like *Aplysia* (Howells, 1942) and *Limnaea* (Carriker, 1946). It has been claimed that an esterase may be present in the saliva of *Helix* (at least in the crop juice; Graetz, 1929), but it is possible that this esterase gets into the crop via the stomach, and is not secreted by the salivary glands.

The midgut gland produces carbohydrases, proteases (including peptidases and perhaps in certain cases also dipeptidases as extracellular enzymes), and esterase (lipase). The amount of enzyme produced by this gland seems to depend more or less on the natural diet and on the amount of food available. We see, therefore, that a profound specialization of the main digestive glands with respect to the enzymes secreted does not seem to occur. This is not surprising, because as a rule the digestive tract is not divided into separate digesting chambers, each with its own task, characterized by its own pH, secretion, etc. In fact, usually there are only one or two chambers (crop and stomach) and of course the intestine proper, in which all the digestive juices are mixed. Or is this only apparently so? Is there perhaps a separation which is not striking, but nevertheless real, and if so, to what extent? In mussels and snails it is well known that the feces appears as a darkish mass on top of which lies a lighter colored, often zig-zag, ribbon. These two fecal excreta are known as the intestinal and the glandular feces. When much food is available, intestinal feces forms the bulk of the material. With little food present, it decreases appreciably in amount. This feces stems from material bypassing the midgut gland and is transported directly into the hind gut. It appears less well digested, and is consequently less well utilized, than the food which gets into the midgut gland. Thus we find here indeed a separation into two main chambers, in one

of which (lumen of the midgut gland) the digestion becomes "complete."

Until lately, it has generally been held that in lamellibranchs the crystalline style contains the only extracellular enzymes. Amylase certainly occurs in this structure. Claims for the existence here of saccharase (van Rijnberk, 1908), butyrase (Sawano, 1929), lipase (George, 1952), glucosidase, and cellulase in *Mya* and in *Mactra* (Lavine, 1946), seem to be questionable at the moment. These enzymes may be of bacterial origin. But Mansour (1946) and Mansour-Bek (1948) found protease, peptidases, and esterase in the stomach juice of *Tridacna pinctada* and *Unio prasedens*. These would be secreted by the digestive diverticula (Mansour and Zaki, 1946). According to the authors, these enzymes cannot originate from bursting or cytolizing amebocytes because their strength in the stomach juice is much greater than that found in extracts made from concentrated amebocytes.

Albrecht (1920) found, in the stomach and intestine of abalone, owl limpet, *Cryptochiton*, and *Ischnochiton*, amylase, emulsin, lactase, lipase, maltase, "pepsin," and saccharase. Since the midgut gland contains the same enzymes (only in that of *Cryptochiton* amylase was apparently lacking), it is possible that the enzymes found in stomach and intestine are secreted by the midgut gland. They certainly do not originate in the food.

Zaki (1951) found proteases, peptidases, and lipase in the stomach juice of *Unio prasedens*. Because the same enzymes could be extracted from the digestive diverticula, and because there was no evidence that they originated from amebocytes, he concluded that they were secreted by the diverticula. It seems, therefore, that we will have to accept an extracellular (pre-) digestion in lamellibranchs, although an intracellular digestion certainly occurs also (Vonk, 1924).

### RESORPTION AND PHAGOCYTOSIS

This brings immediately to the fore the problem of resorption, of which surprisingly little is actually known in molluscs. Where and how does the active uptake of (dissolved) digested substances occur? Jordan and Begemann (1921) did not find a true resorption of fluid (coupled with expenditure of energy) in the intestine of *Helix pomatia* when it was filled with a sugar solution. A diffusion of sugar did, however, occur through the intestinal wall.

True resorption, often combined with phagocytosis, seems to occur in the midgut gland. Hirsch (1915) observed in the live *Pterotrachea*, a predigestion in the crop, into which digestive juice from the midgut gland is poured. After about four hours the predigested food is moved into the midgut gland where the final digestion and resorption take place. Undigestible remains are then passed on to the hind gut, where apparently water is resorbed (formation of fecal pellets). In *Helix pomatia* (unpublished) and in *Achatina fulica* the uptake of iron saccharate was followed (van Weel, 1950). In the cells of the midgut iron appears only diffusely distributed. There is no indication of a granular concentration. This suggests a diffusion here, not a true resorption, as Jordan and Begemann had pointed out already. In the cells of the midgut gland the iron appears first diffusely distributed in the apical cytoplasm, after which an active granular concentration occurs (Fig. 1b). The iron granules, being undigestible, are finally extruded with the secretion.

In the aeolid *Gratena* iron appears also in definite granular form in the epithelium of the stomach (Graham, 1937/1938); this suggests a true resorption (Fig. 1a). Yonge (1926) demonstrated an apparently true resorption of iron saccharate in the stomach of the oyster. Whether this is a general feature in lamellibranchs or an isolated case cannot be decided at the moment.

In gastropods the midgut gland also phagocytizes. Intracellular digestion follows the uptake of particulate matter. The problem of whether phagocytosis really occurs in this gland has been hotly contested. Indeed, the methods and techniques employed are often not decisive: India ink and carmine, most often used as tracers in these

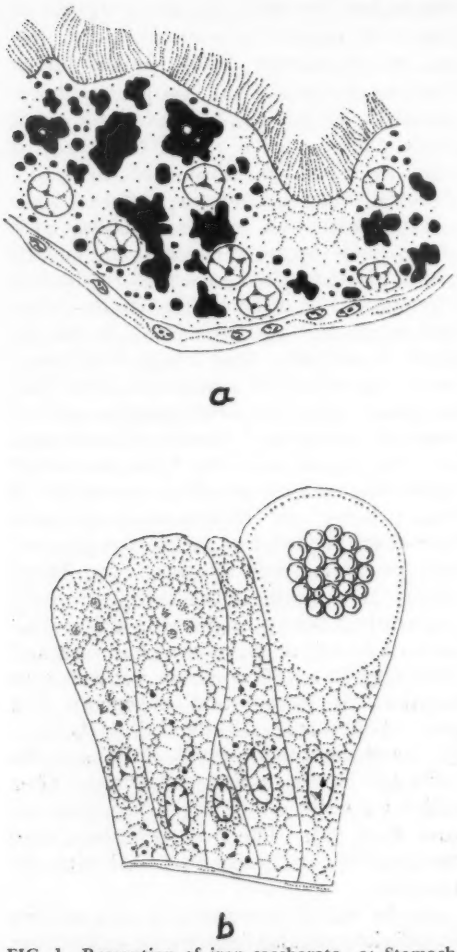


FIG. 1. Resorption of iron saccharate. a: Stomach epithelium of *Gratena* (after Graham 1937/38, from von Buddenbrock 1956); b: Midgut gland of *Achatina*. 2 hrs. after feeding started (after van Weel 1950).

investigations, do not guarantee the occurrence of phagocytosis, because part of the substances exist also in colloidal, semicolloidal, even true molecular solution (Krijgsman, 1928). Thus the appearance of black or red granules in the cells may be caused by diffusion or resorption, followed by granular concentration in the cytoplasm, and not by phagocytosis per se. However, it may now be considered an established fact that phagocytosis does occur in the digestive diverticula of *Mytilus*, *Ostrea* (Vonk, 1924), *Teredo* (Potts, 1928), in the midgut gland of *Limnaea* (Peczenik, 1925), *Helix* (Rosén, 1942), and *Achatina* (van Weel, 1950). But phagocytosis does not occur at the same rate in the various species: the "willingness" to phagocytize may differ markedly. Furthermore there often seems to be a definite selectivity of the cells with respect to what particles will be phagocytized: completely undigestible substances are often rejected, but when they are coated with a digestible layer they are phagocytized readily (Peczenik, 1925, Rosén, 1942). This is probably the reason for the conflicting observations and conclusions. The only molluscs which apparently do not show any phagocytosis are the cephalopods. However, Bidder (1957) claims to have found evidence for phagocytosis in the "liver" of *Octopus*. Since he used carmine in his experiments, his conclusions appear questionable. Further investigation is urgently needed to clarify this point.

The general conclusion seems to be that intracellular digestion does or can occur in the digestive diverticula of the midgut gland in the majority of molluscs; that (probably with the exception of cephalopods) these organs are the main seat of resorption; and, so far as evidence exists, that the intestine itself does not show a true physiological resorption, although diffusion may occur here. However, particularly on this last point, more investigations are badly needed.

## "FITNESS" OF ENZYMES

It is the general conception that the physiology of digestion is "linked" to the normal diet of animals: enzyme equipment and secretion "fit" the diet. Recently this problem has been investigated in *Achatina fulica*, an omnivorous snail when mature. Prosser and van Weel (1958) kept adult snails either on a carbohydrate-rich (boiled potatoes), or a protein-rich diet (lean horse meat) for a period of four weeks. Each week of this period the cathepsin, esterase, and amylase activity of the midgut gland

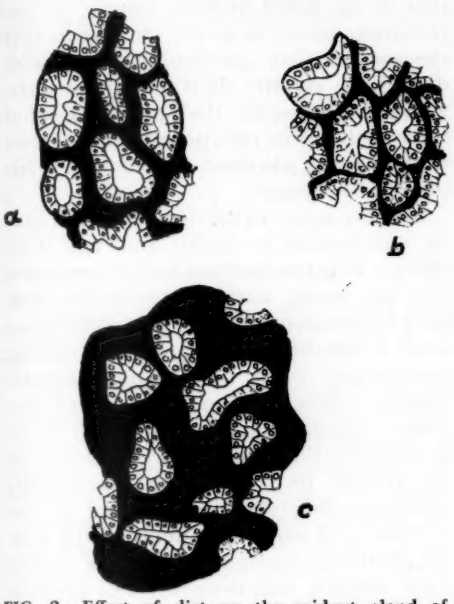


FIG. 2. Effect of diet on the midgut gland of *Achatina*. a: Starved; b: protein diet; c: starch diet. b and c after 8 weeks.

were determined. No significant differences, due to the special diets, were found in cathepsin and esterase activity between the two groups, but quite an unexpected change in amylase activity was found: the protein-fed snails appeared to produce more amylase than the starch-fed ones. The experiments were later repeated over a longer period of time (eight weeks) with a slightly different and improved method and technique (van Weel, 1959): instead of computing the enzyme activity on a basis of a unit of dry-weight gland powder, it was now based on a unit of Kjeldahl-N of the gland. Prosser and van Weel had described important histo-morphological changes of the gland as a result of special diets (Fig. 2). Such changes could very well affect the dry-weight of the gland powder. In these experiments it was found that the protein-fed snails produce less protease and less cathepsin than the starch-fed animals (Fig. 3). Thus the proteases did not fit the diet. Contrary to the earlier observations, amylase and saccharase did "follow" the diet: starch-fed snails seem to produce more carbohydrases than the protein-fed ones (Fig. 4). The discrepancies between the results of the two investigations must probably be sought in the different techniques used. No explanation can be offered as to why protease and cathepsin do not "adapt" to the diets.

The effect of the diets on the utilization of the food, i.e., the amount of food resorbed, were also investigated (van Weel, 1959). The results (Fig. 5) do not run parallel to the enzyme strength of the midgut gland. Furthermore, the starch-fed snails

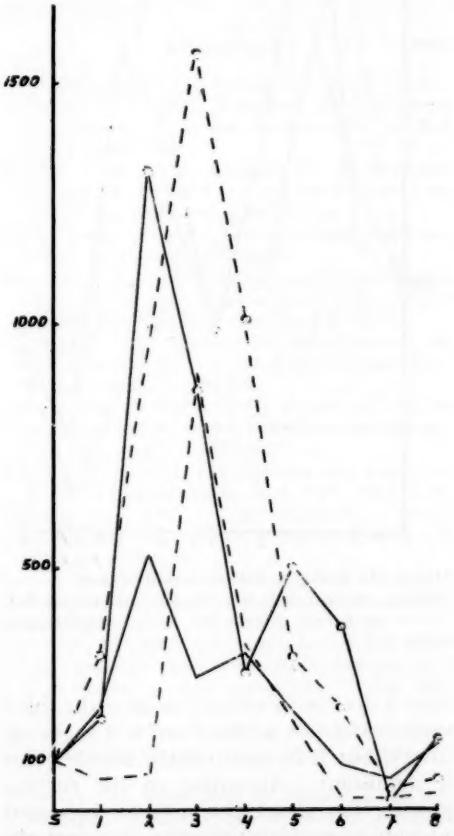


FIG. 3. Enzyme strength in percentage of that of starved snails (= 100), based on aliquots from extracts of 100 mg Kjeldahl-N/g gland powder. ——— protease, protein fed; o——o protease, starch fed; - - - - - cathepsin, protein fed; o- - - - -o cathepsin, starch fed. S: starved snails. Abscissa: weeks of dieting.

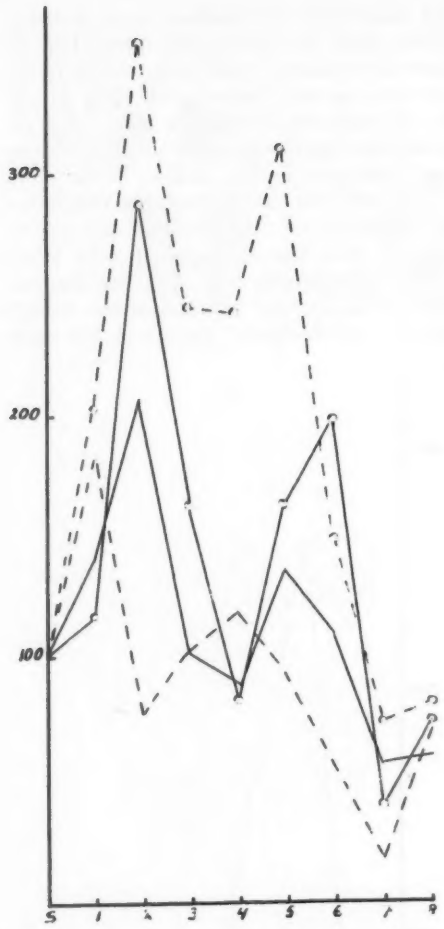


FIG. 4. As in Fig. 3, but for carbohydrases. ——— amylase, protein fed; o——o amylase, starch fed; – – – – saccharase, protein fed; o– – – –o saccharase, starch fed.

show a decline in utilization after the third week. After six weeks there is a slight recovery, but it is questionable whether this is significant. According to the enzyme picture, the gland produces an increased amount of amylase between four and six weeks, when the utilization of the food is lowest. The protein utilization declines also after three weeks, but a recovery is apparent at five weeks. Again this does not match the protease and cathepsin production in the gland at these times. An explanation cannot be given. It appears that the special diets (particularly the starch diet) lack certain dietary requirements, which may cause the decline in utilization (very probably in resorption). Meat seems to be a more adequate diet for the snails than boiled potato.

Another aspect of the digestion in *Achatina* was studied by Smith and van Weel (1960). It is known from field observations that the young, immature snails are typically herbivorous. They turn omnivorous (with a definite leaning towards becoming carnivorous) when they become sexually mature. Here we have an animal that shows a natural change in diet. It could be expected that this would be reflected in the enzyme picture. Indeed, statistically significant differences were found in the protease and amylase contents of the midgut gland: the young snails produce slightly more amylase and definitely less protease than the mature ones (Table 2). In this case the enzyme picture "fits" the feeding habits. Whether the change in the enzyme pattern affects or causes the food preferences, or whether it is only part of the entire physiological processes in the snail which lead to a change in feeding habits, must be left undecided.

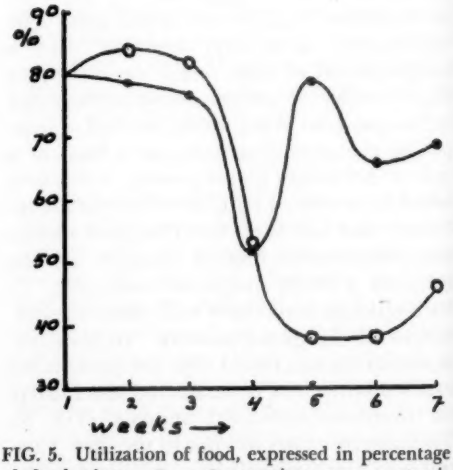


FIG. 5. Utilization of food, expressed in percentage of food given. •——• proteins; o——o starch. Abscissa: weeks of dieting.

TABLE 2. *(after Smith and van Weel)*

| Amylase | | Protease | |
|---|---|---|---|
| Young snails | Mature snails | Young snails | Mature snails |
| 70.4* | 70.4* | 0.02† | 0.22† |
| 74.4 | 70.4 | 0.15 | 0.21 |
| 73.1 | 69.2 | 0.15 | 0.17 |
| 73.2 | 70.4 | 0.09 | 0.21 |
| | 70.4 | 0.10 | 0.18 |
| | | | 0.26 |
| | | | 0.18 |

*Amount of reducing sugars present, expressed in mg glucose.

† Sörensen's Formaldehyde titration of 2 ml of enzyme-substrate mixture.

Nothing has been mentioned yet of the existence of a rhythmic extrusion of digestive secretions, because this belongs more to a specific gland physiology (although it will have an effect on the digestion, of course). As a matter of record it may be pointed out that if a rhythm exists in the gland it might be possible to determine what cell types produce which enzymes. This has been tried (van Weel, 1949, 1950, 1960), but to no avail. Without question rhythms do exist, but they seem to be irregular (see also Postma et al., 1945). Whether such rhythms affect the feeding habits is a problem that has not yet been investigated, but may be interesting to pursue. Nothing is known of rhythms in the digestive diverticula of lamellibranchs. However, we do know of a rhythmic opening and closing of shells of oysters, etc., even in perfectly clear water. It would be of interest to find out whether there is a correlation between these movements and the activity of the digestive diverticula.

## CONCLUSIONS

In this short review of the physiology of digestion in molluscs only a comparatively few problems have been discussed. It is clear that not much is known about many aspects of digestion: practically nothing is known of the permeation through the intestinal wall in the various groups, and research in this field is urgently needed. What is the correlation between "willingness" to phagocytize and true resorption? Does the midgut gland "take over" from the intestine, or is it the other way around? What are the causes of apparent changes in resorption and enzyme production, due to drastic changes in the diet? Is there an adaptation in the long run? We have only indications of a correlation between utilization and amount of food available; we lack definite data. What about the effect of age on enzyme production, resorption, and utilization? These and many more problems present themselves to everyone who turns to the molluscs for the study of the physiology of digestion, problems which await solution.

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# AMINO ACIDS IN THE MOLLUSCA

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The subject first suggested for this review was "Nitrogen Metabolism in the Mollusca." After examining the literature it seemed more appropriate to consider one of the basic groups of compounds in nitrogen metabolism: namely, the amino acids. There were several reasons for this. One, investigations dealing with the amino acids in Mollusca have not been reviewed, and these studies date back to 1845. Two, the role of amino acids as osmoregulators has been suggested for some time, and recent work on molluscs strongly supports this idea. Three, many areas of nitrogen metabolism in the Mollusca have not been studied. A considerable amount of work has been done on nitrogen catabolism, but even here the major problem of the biosynthesis of uric acid is yet unsolved. This paper therefore will discuss the work dealing with amino acid constituents of molluscan tissue, physiological studies involving amino acids, and the enzyme systems which have been studied that are pertinent to amino acid metabolism in the Mollusca.

## AMINO ACIDS IN MOLLUSCAN TISSUE

The identification of "free" amino acids (i.e., in contrast to those bound in protein) is not a new phenomenon. Chemical isolation and quantification of several amino compounds was accomplished in the 19th century. One of the amino acids isolated from molluscan tissue was taurine, a sulfonic amino acid. Not only was this compound found in a number of molluscan species, but it was also found to be present in high concentration. The first report of this amino acid is recorded in a paper by Mendel and Bradley (1906). "The occurrence of taurin in the muscle of invertebrates has been known since 1845, when Karsten isolated a crystalline body from the watery extracts of certain molluscs, and identified it qualitatively with the taurin of ox bile." Later workers reported taurine from all of the major classes of the Mollusca. Krukenberg et al. (1885), Henze (1905, 1913, 1914), Suzuki et al. (1909), Okuda (1920) and Ackerman et al. (1924) found large quantities of taurine in cephalopods. Kelley (1904) extracted glycine and taurine from several species of invertebrates. *Mytilus edulis,* one of the species examined, was found to contain 1.6% taurine based on dry weight of tissue. Mendel (1904), Mendel and Bradley (1906), and Schmidt (1917) have likewise reported taurine from gastropods. It is of interest that all of the above investigators used marine species in their studies.

Other componds, such as glycine and arginine, were also isolated by chemical means. Chittenden (1875) obtained pure glycine from the muscle of *Pecten irradians.* Ackerman (1922) reported arginine from *Mytilus edulis.* In the same year Ackerman et al. isolated adenine and arginine from the muscle of *Eledone moschata,* a cephalopod. Arnold and Luck in 1932 examined several species of vertebrates and invertebrates for arginine and arginine phosphate. High concentrations of both of these compounds were found in the tissues of the cephalopod *Paroctopus apollyn.* This latter study followed the work of Kutscher and Ackerman (1926) which indicated that arginine and arginine phosphate are present only in invertebrates. Creatine and creatinine phosphate were isolated from vertebrates and echinoderms. Reviews on this subject have been given by Baldwin (1933) and more recently by Ennor and Morrison (1958). No further discussion of the phosphagens will be given here, except to point out that the distribution of creatine phosphate in the animal kingdom is not nearly as limited as the earler workers suggested.

The advent of microbiological assay and paper chromatography made possible more complete analyses of amino acids in animal tissue. Noland (1949) determined by mi-

crobiological assay some of the amino acids in the pelycypod *Mactra solidissima*, the gastropod *Buscycon canaliculatum*, and the cephalopod *Loligo peali*. Relatively high values for tryptophane, tyrosine, and histidine were recorded for *L. peali*, while only traces of these compounds were found in the other two species. Duchâteau and Florkin (1954), by the same method, determined values for fifteen amino acids in the foot of the marine gastropod *Buccinum undatum*. Alanine, arginine, glycine, and proline were the amino acids present in highest concentrations. Ramamoorthi (1958) investigated the amino acid composition of developing snail eggs. The eggs of *Pila virens* were extracted during various stages in development and amino acids were quantitated. In the one-cell stage only faint traces of alanine, threonine, glutamic acid, aspartic acid, serine, and glycine could be detected. In the two-cell stage hydroxyproline appeared, but disappeared in the three-cell stage. As development continued, the concentration of the amino acids increased. In the larval stage three other amino acids were detected: leucine, isoleucine, and phenylalanine. Kavanau (1958) in a similar study reported values for "free" valine during the development of *Spisula solidissima* eggs. From fertilization to the third cleavage stage there was a gradual increase in "free" valine. There was no marked increase or decrease of this compound during subsequent division cycles. With regard to these studies on amino acids in developing eggs, it should be pointed out that the first report of "free" hydroxyproline in molluscan tissue was that of Ramamoorthi. This compound had been found in molluscan collagen by Melnick (1958) and Williams (1960) following hydrolysis. In view of Kavanau's (1958) study on autolysis, the report of hydroxyproline as a "free" amino acid needs verification.

Simpson et al. (1959) by the use of paper chromatography examined the amino acid content of numerous aquatic invertebrates. Their results on the molluscan species were interesting. The sulfonic amino acid taurine, which was found in high concentration by the earlier investigators, was detectable only in marine forms. This compound was not detectable by paper chromatography in the fresh water and terrestrial species. Their results are shown in Table 1. In this same study a $\beta$-amino acid, $\beta$-alanine, was found in four species of pelecypods and in the cephalopod *Loliguncula brevis*. In the same year Awapara and Allen isolated $\beta$-aminoisobutyric acid from *Mytilus edulis* and *Volsella dimissus*. These $\beta$-amino acids are the products of thymine and uracil catabolism in mammalian tissue and thus may be of particular interest for future studies in pyrimidine metabolism in molluscs.

TABLE 1. *Taurine content in various molluscs*

| Species | Environment | Taurine |
|---|---|---|
| **Gastropoda** | | |
| *Lymnaea palustris* | fresh water | — |
| *Marisa cornuarietis* | fresh water | — |
| *Pomacea bridgesi* | fresh water | — |
| *Rumina decollata* | terrestrial | — |
| *Otala lactea* | terrestrial | — |
| *Mesodon thyroidus* | terrestrial | — |
| *Bulimulus alternatus* | terrestrial | — |
| *Murex fulvescens* | marine | + |
| *Littorina irrorata* | marine | + |
| *Oliva sayana* | marine | + |
| *Polinices duplicata* | marine | + |
| *Buscycon perversum* | marine | + |
| *Siphonaria lineolata* | marine | + |
| *Fasciolaria distans* | marine | + |
| *Thais haemastoma haysae* | marine | + |
| **Pelecypoda** | | |
| *Anadonta grandis* | fresh water | — |
| *Quadrula quadrula* | fresh water | — |
| *Lampsilis* sp. | fresh water | — |
| *Elliptio* sp. | fresh water | — |
| *Rangia cuneata* | brackish–fresh water | — |
| *Brachiodontes recurvus* | brackish–marine | + |
| *Crassostrea virginica* | brackish–marine | + |
| *Donax variabilis* | marine | + |
| *Venus mercenaria* | marine | + |
| *Dosinia discus* | marine | + |
| *Arca incongrua* | marine | + |
| *Arca campechiensis* | marine | + |
| *Noetia ponderosa* | marine | + |
| **Cephalopoda** | | |
| *Loliguncula brevis* | marine | + |

Since taurine was detectable only in the marine molluscs, Allen and Awapara (1960) compared the metabolism of sulfur amino acids in two species of pelecypods. They

studied the metabolism of S-35 methionine in the marine mussel *Mytilus edulis* and in the brackish-water clam, *Rangia cuneata*. Taurine was not detectable in the latter. They found that both species could convert methionine to cysteine, probably in the same manner as do mammals, and that cysteine was oxidized to products which could give rise to taurine. Two differences were observed between the two species. One was in the mechanism of oxidation of cysteine to taurine. In *R. cuneata* large amounts of labelled cysteic acid were detected, indicating that cysteine sulfinic acid is oxidized to cysteic acid and the latter then decarboxylated to taurine. In *M. edulis* cysteic acid was not formed, but another compound, hypotaurine, appeared as a labelled intermediate. Hypotaurine (2-aminoethanesulfinic acid), as reported by Chatagner et al. (1951), Bergeret et al. (1952a and 1952b), and Awapara and Wingo (1953), may be the main intermediate from cysteine sulfinic acid to taurine in the rat. This compound is formed by the decarboxylation of cysteine sulfinic acid. Hypotaurine has been reported from molluscan tissue by Shibuya and Shunji (1957) and Ouchi (1959). Their results were similar to those of Robin and Roche (1954), who, working on coelenterates and sponges, detected hypotaurine in those species in which a high concentration of taurine was also present. Hypotaurine has never been observed in vertebrate tissue except in in vitro studies where the precursor has been supplied.

The second difference between *M. edulis* and *R. cuneata* was in the disposition of taurine. In *M. edulis* taurine was formed and retained in the tissue while in *R. cuneata* taurine was formed but within a few hours disappeared from the organism. A summation of the metabolism of methionine in *M. edulis* and *R. cuneata* is shown in Fig. 1. To extrapolate these results to those of Simpson et al. (1959), one may suggest that the absence of taurine in the freshwater and terrestrial species is due to the rapid disappearance of the compound from the tissue rather than an inability to to synthesize it. These authors suggest that taurine *then* might play a role in osmoregulation in the marine forms.

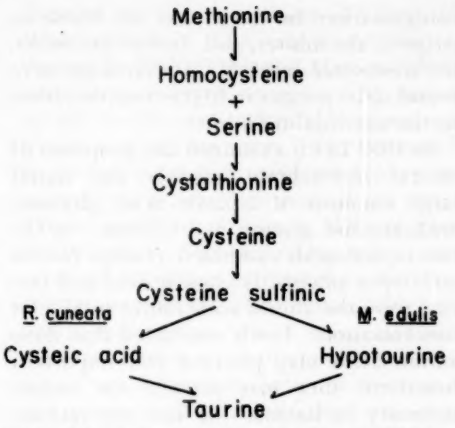


FIG. 1. Metabolism of S-35 methionine by *Mytilus edulis* and *Rangia cuneata*.

### AMINO ACIDS AND OSMOREGULATION

Associated with the work on identification of amino acids have been investigations relating amino acid concentration in an organism with the salinity of its environment. Duchâteau et al. (1952) compared the amino acid concentration in the marine forms *Mytilus edulis* and *Ostrea edulis* with that of the freshwater species *Anodonta cygnea*. The results shown in Table 2 indicate that the freshwater form has a lower concentration of amino acids than the marine species. Similar results have been obtained when comparisons of amino acid concentrations in fresh water and marine crustaceans were made. Camien et al. (1951) reported differences in amino acid

TABLE 2. *Free amino acids*

| | mg/100 g of tissue water | | |
|---|---|---|---|
| Amino acid | *M. edulis* | *O. edulis* | *A. cygnea* |
| Alanine | 340 | 646 | 8.8 |
| Arginine | 415.5 | 66.6 | 36.5 |
| Aspartic acid | 200.4 | 26.1 | 4.4 |
| Glutamic acid | 317 | 264 | 29.4 |
| Glycine | 399 | 248 | 13.2 |
| Histidine | 12.1 | 22.9 | 2.5 |
| Isoleucine | 24.8 | 19.2 | 6.3 |
| Leucine | 15.4 | 12.9 | 3.6 |
| Lysine | 39.4 | 22.0 | 8.2 |

concentration in the tissues of *Homarus vulgaris,* the lobster, and *Astacus fluviatilis,* the freshwater crayfish. Amino acids were found to be present in higher concentration in the sea-dwelling lobster.

In 1952 Lewis examined the axoplasm of several invertebrate animals and found large amounts of aspartic acid, glutamic acid, alanine, glycine, and taurine. In the two cephalopods examined (*Loligo forbesi* and *Sepia officinalis*) aspartic acid and taurine were the amino acids found in highest concentration. Lewis concluded that these amino acids may perform two important functions: they may provide the anions necessary to balance the internal cations; they may also provide extra solutes necessary for osmoregulation. In view of this high concentration of free amino acids in squid axoplasm, Koechlin (1954, 1955) attempted to establish an acid-base balance in squid axoplasm. He isolated and identified a substance responsible for a large portion of the organic anions. This substance was found to be a hydroxy derivative of taurine, isethionic acid (2-hydroxyethane sulfonic acid). This was the first report of this compound in biological material. A review of the studies on squid axoplasm up to 1957 has been given by Schmitt and Geschwind. Two more recent papers dealing with the constituents of axoplasm are those of Deffner and Hafter (1959a and 1959b). Continuing the studies on the organic constituents of squid axoplasm, they found isethionic acid in high concentration in *Loligo peali* and *Dosidicus gigas.* They also quantitated the amino acids present in the axoplasm of these two species. They found that four of the amino acids, arginine, lysine, ornithine, and taurine, were lower in concentration in *D. gigas.* Further study revealed the presence of a peptide in relatively high concentration. On isolation and hydrolysis this peptide was found to contain cysteic acid amide. This was the first report of such a compound in biological material. This also increased the number of sulfur-containing compounds in axoplasm to four: methionine, taurine, isethionic acid, and cysteic acid amide. The method of biosynthesis of the latter three compounds in axoplasm is unknown.

The role of amino acids in axoplasm as osmoregulators is now fairly well established. The suggestion that amino acids may play such a part in osmoregulation is not new. Fredericq (1904) examined several invertebrates with regard to their inorganic constituents in tissue. He found the inorganic ion concentration in muscle and certain other tissues was considerably lower than in the blood, where the inorganic concentration was similar to that of the surrounding sea water. He therefore postulated that these tissues contain relatively large amounts of small organic molecules. It is of interest in this connection that the early investigators mentioned in the previous section had already found high concentrations of glycine and taurine in numerous species of marine invertebrates, including several species of molluscs.

Studies establishing amino acids as osmoregulators in molluscan tissue have not been as convincing as those on squid axoplasm. Potts (1958) studied the effect of changing salinity on *Mytilus edulis* and *Anodonta cygnea.* He noted that the potassium and chloride of the muscle tissue were not in a Donnan equilibrium with the potassium and chloride ions of the blood. Further, *M. edulis* adapted to a dilute environment by an increase in water content, a reduction in the concentrations of sodium and chloride ions, and a reduction in the amino acid concentration in muscle fibers. The amino acid concentration was measured as amino nitrogen. *Anodonta cygnea* adapted to increased salinity by a reduction in water content and an increase in sodium and chloride ions in muscle fibers. Allen (1959, unpublished data) observed an increase in the amino acid content of *Rangia cuneata* (a brackish-water clam) as it gradually progressed from salinities of 3 ‰ to 25 ‰. This work was not a study in osmoregulation, but rather an investigation on the effect of salinity on the concentration and/or production of taurine. The earlier study of Simpson et al. (1959) had shown that only the marine Mollusca had taurine in

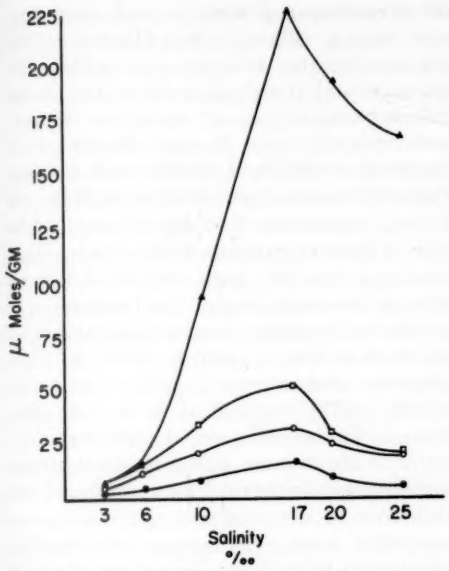


FIG. 2. Concentration of four amino acids in *Rangia cuneata* from different salinities. ▲—alanine; □—glycine; ○—glutamic acid; ●—aspartic acid. The amino acid concentration was based on dry weight of tissue.

concentrations high enough to be detected by paper chromatography. The result of moving *R. cuneata* to higher salinities was not an increase in taurine to detectable quantities, but rather a rapid increase in the concentration of alanine. The results of Allen's study are shown in Fig. 2. One of the problems involved in the increase of amino acids with an increase in salinity is the source of these compounds. Potts (personal communication) in studying the effect of salinity changes on the amphipod, *Gammarus zoddeckei,* observed an increase in the amino nitrogen as the animal passed into higher salinities. The reverse was true when this species was subjected to more dilute environments. Potts thought that the animal increased its amino nitrogen by hydrolyzing protein. If such were the case in *R. cuneata,* it is difficult to understand how only one amino acid such as alanine would increase in concentration. Allen suggests that protein hydrolysis is just one part of the mechanism, and feels that as the animal encounters a change in salinity there is also an expenditure of energy for osmoregulation. This was assumed to be the case by Pieh (1936) and Maloeuf (1938) when they observed an increase in oxygen uptake in *M. edulis* when this mussel was placed in dilute sea water. The increase in oxygen consumption reappeared when the animal was returned to normal sea water. This expenditure of energy, Allen suggests, would supply short-chain keto acids which could be utilized as substrates in the transamination reaction. The amino acids from hydrolyzed protein would serve as amino donors. If one particular transaminating system should predominate, the increase of one amino acid over the others would be possible. For example, pyruvic acid is the amino acceptor in the formation of alanine and is also an end product of anaerobic glycolysis. Transaminase studies on molluscan tissue have been done by Roberts (1958) in which the glutamic-aspartic system was reported to be present in octopus axoplasm. Wilbur (1960) incubated shell preparations of *O. edulis* in C-14 labelled $NaHCO_3$ and proprionic acid. The study was concerned with carbon dioxide fixation, but in the process of fixation some of the labelled carbon appeared in amino acids. Wilbur suggests that the labelling occurred via transamination, since the incorporation of carbon dioxide proceeds through keto-acids of the Krebs cycle. Allen (1959, unpublished data) has also found the glutamic-aspartic transamination reaction to be active in the hepatopancreas of *Otala lactea, Lymnaea palustris, Marisa cornuarietis,* and *Pomacea bridgesi.*

### ENZYMES IN AMINO ACID METABOLISM

*Amino acid oxidases.* The first record of a D-amino acid oxidase in molluscs was reported by Blaschko and Hawkins (1951). The study was made with tissue homogenates, and racemic mixtures of amino acids were used as substrates. Activity was demonstrated on the basis of oxygen consumption. The organ used in this study was the digestive gland of *Octopus vulgaris* and *Sepia officinalis.* Blaschko and Hawkins (1952) reported the presence of what they

thought was the enzyme previously found in cephalopods in *H. pomatia, M. edulis,* and *Anodonta sp.* Although the results were positive for the three species, a considerable difference in activity as well as substrate selectivity was observed among the three species. In all cases racemic mixtures of amino acids were used. In 1952 Roche et al. (a and b) described an enzyme in the hepatopancreas of *M. edulis* that oxidized L-arginine. The oxidation products obtained in vitro and in vivo were γ-guanidobutyric and α-keto δ-guanidovaleric acids. These results prompted Blaschko and Hope (1956) to re-examine *M. edulis* in order to determine whether a D- or L-amino acid oxidase was present. By the use of a manometric technique they found that the enzyme was an L-amino acid oxidase which acted on basic amino acids, particularly arginine. Robin et al. (1957) reported what is apparently the same enzyme from *Lymnaea stagnalis.* By means of paper chromatography they isolated the oxidation products reported earlier by Roche. Thoai et al. (1954) investigated the metabolism of L-histidine in *M. edulis.* The products obtained following incubation of hepatopancreas tissue with L-histidine were imidazolpyruvic and imidazolacetic acids. Further degradation of the side chain on the imidazol nucleus of histidine resulted in the appearance of imidazolmethanol and imidazolmethanal. The above compounds were identified by means of paper chromatography. The ability of this tissue to degrade histidine beyond imidazolpyruvic and imidazolacetic acids testifies to the existence of other enzymatic processes which need further studies. Blaschko and Himms (1955) reported another D-amino acid oxidase, D-glutamic and D-aspartic acid oxidase, from the hepatopancreas of *Octopus vulgaris* and *Sepia officinalis.* Recently Roche (1959) reported a soluble L-amino acid oxidase from *Cardium tuberculatum* which required magnesium ions for activity. The role of these enzymes in molluscan metabolism has not yet been determined. Although the validation of the D-amino acid oxidase is necessary, a general summary of the work to date is given in Table 3.

*Arginase and nitrogen catabolism.* Clementi (1915) demonstrated, for the first time in an invertebrate, the enzyme arginase. This enzyme, acting on its substrate arginine, produced urea and ornithine. In this work he surveyed many animal species including some vertebrates; only one invertebrate, *Helix pomatia,* contained arginase. It was on the basis of this investigation that Clementi used the words ureotelic and uricotelic referring to the excretion of urea and uric acid respectively. The presence of arginase, according to Clementi, was an indication of ureotelism. The absence of arginase would, conversely, indicate uric acid excretion. Strohl (1924), reviewing the literature of previous years, referred to works in which high concentrations of uric acid in molluscs, including *H. pomatia,* were reported. Later Hunter et al. (1925) examined a starfish, *Pisaster ochraceus,* a crab, *Cancer productus,* and a clam, *Saxidomus giganticus,* for arginase, but found none. They concluded that *H. pomatia*

TABLE 3. *Amino acid oxidases in the mollusca*

| Enzyme | Species | Investigator |
|---|---|---|
| D-amino acid oxidase | *H. aspersa*<br>*S. officinalis*<br>*O. vulgaris*<br>*Anodonta* sp. | Blaschko and Hawkins, 1951, 1952 |
| L-amino acid oxidase | *M. edulis*<br>*L. stagnalis*<br>*C. tuberculatum* | Blaschko and Hope, 1956<br>Roche et al., 1952, 1957, 1959<br>Thoai et al., 1954<br>Robin, 1957 |
| D-glutamic acid oxidase<br>D-aspartic acid oxidase | *L. forbesi*<br>*O. vulgaris*<br>*E. cirrosa* | Blaschko and Himms, 1955 |

was an exception to Clementi's rule, for although arginase was present uric acid was excreted in large amounts by this species. Albrecht (1923) and Delauney (1931) examined numerous species of invertebrates and reported traces of urea in their tissues and urine. In *H. pomatia* urea was found in small amounts in the tissues. Delauney also reported values for amino nitrogen in molluscan urine, indicating that these compounds were excreted by numerous species.

In view of the values reported for urea and the presence of arginase in *H. pomatia,* Baldwin and Needham (1934) worked on this snail in hopes of demonstrating the ornithine cycle of Krebs (1932). If this cycle should be present, Clementi's hypothesis that arginase and ureotelism were related would be supported. Their findings were confusing, for the only source of urea appeared to be the arginine in the food. Ornithine incubated with tissue slices did not increase the amount of urea above the normal level for the tissue involved, nor did the incubation of ornithine and arginine together produce more urea than arginine alone. Since there were no numerical values given for arginase in *H. pomatia,* Baldwin (1935a) reinvestigated the question of urea synthesis in this snail, and his results showed arginase activity to be as high as that reported for mammalian liver. Baldwin then postulated that urea was synthesized in the snail, but that its low level indicated that it was metabolized to some other compound as fast as it was formed. This problem of urea and uric acid in molluscs was investigated from another angle by Needham (1935), who showed that a high concentration of uric acid was associated with a terrestrial environment in snails. Baldwin in the same year compared the concentration of uric acid in several species with the activity of arginase and observed that there was a direct correlation between the level of arginase and uric acid concentration. This suggested to Baldwin that arginase played a role in uric acid formation. Baldwin (1935b) investigated the possibility that uric acid may be formed by the condensation of urea with hydroxymalonic acid and tartronyl urea as suggested by Wierner (1902). Although positive results were obtained the question of uric acid synthesis is still open. The more recent studies have simply increased the number of species examined for uric acid rather than investigating the biosynthesis of this compound. Lal et al. (1952) and Saxena (1956) reported concentration for uric acid in *Pila globosa,* the Indian apple snail, and Meenakshi (1955) for *Pila virens.* Saxena (1953) reported arginase activity in *Achitina fulica.*

## SUMMARY

Many problems remain unanswered in the area of amino acid metabolism in the molluscs. In fact, it is almost impossible with the present status of information to do much as far as conclusions and hypotheses are concerned. There are two areas of investigation which have been studied to some extent. One is the role of amino acids in osmoregulation, and the other is the biosynthesis of uric acid in land snails.

As far as osmoregulation is concerned, it appears that taurine plays an important role in the marine molluscs. However, the amino acids in general are found in higher concentration in marine species than in the fresh water forms. It may be that taurine acts as an osmoregulator in some species such as *M. edulis* (taurine is 1.6% of the dry weight). In other species a different amino acid may assume this role. Glycine and alanine have been found in high concentration in several species. Much of the survey work in quantitating amino acids has been done. The questions of how these animals effectively maintain high concentrations of particular amino acids and the source of these compounds have yet to be answered.

The biosynthesis of uric acid in land snails is another unsolved problem involving the metabolism of amino acids. Although some of the excreted uric acid results from nucleic acid catabolism, the amount found in land snails is above that which would be expected if only nucleic acids were involved. Evidence suggests

that arginase, an enzyme involved in the breakdown of arginine to urea and ornithine, may be associated with uric acid production. The experimental evidence for this needs verification.

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# COMPARATIVE FUNCTIONAL MORPHOLOGY OF BORING MECHANISMS IN GASTROPODS

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Organisms capable of perforating the calcareous exoskeleton of other organisms are represented in the algae, fungi, bacteria, and in at least 7 animal phyla. Better known genera of the latter are: the sponge *Cliona,* the flatworm *Pseudostylochus,* the bryozoan *Ctenostomata,* the phoronid *Phoronis,* the polychaete *Polydora,* the barnacle *Trypetesa,* the pelecypod *Lithophaga,* and the gastropod *Natica.*

The mechanism by which penetration is effected is incompletely understood. Among plants and lower invertebrates it may be achieved by chemical means alone; in higher invertebrates, by mechanical, chemical, or a combination of these. Various hypotheses suggest that release of $CO_2$, mineral acids, organic acids, enzymes, or sequestering agents may bring about dissolution of shell. A current theory on formation of dental caries in humans postulates that proteolytic-chelating substances released by bacteria and/or fungi may cause simultaneous dissolution of the organic matrix and enamel apatite of teeth. In higher invertebrates mechanical devices such as valve edges in pelecypods, radulae in gastropods, and bristles in polychaete and sipunculid worms and in barnacles may be employed alone in penetration of soft calcareous substrates; but perforation of hard exoskeletons probably requires chemo-mechanical means. The degree of similarity of the chemical mechanism for shell dissolution in this wide assortment of organisms can only be conjectured at this time. Descriptive and experimental studies in progress by the writer on boring mechanisms in muricid, thaidid, and naticid prosobranch gastropods contribute further information on boring mechanisms and support a chemo-mechanical theory of penetration in which abrasive action of the radula removes shell softened by secretion from the accessory boring organ, the ABO.

It has been recognized for at least 2,300 years that certain predatory gastropods obtain food through holes drilled by them in the calcareous exoskeletons of prey. At present such boring is attributed to species in 6 families: Cymatiidae, Muricidae, Thaididae, and Naticidae (prosobranchs); and Helicidae and Oleacinidae (pulmonates). Little is known about boring in the Cymatiidae, Helicidae, and Oleacinidae. Members of the families Cassidae, Doliidae, and Tritonidae (prosobranchs) are said to secrete acid which may serve in boring or dissolving echinoderm prey. The method of penetration of the prey is poorly understood and requires further study. In Naticidae the ABO is borne on the ventral tip of the proboscis and was first described by Troschel (1854). Schiemenz (1891) first suggested that this ABO secretes an acid, but this has not been confirmed. Ankel (1937, 1938), by placing freshly cut naticid ABOs against the shell, obtained shallow dissolution in a few hours and postulated the presence of a calcase. The ABO in Muricidae

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was discovered independently by Fretter (1941, 1946), who considered it a pedal sucker, and by the writer (1943) who concluded from behavioral studies that it secretes a shell-softening substance.

Study by us of bore holes collected by the Galathea Expedition in 1950-1952, as well as those drilled by snails in our laboratory, suggests that boring gastropods are present in most of the shallower portions of the oceans to a depth of at least 2,700 meters. The oldest gastropod bore holes which we have been able to identify are some 400 million years old from the Middle Ordovician, and suggest the antiquity of the origin of the boring mechanism.

To date 29 different species of boring and several species of non-boring marine predatory gastropods from 21 different areas of the world (including England, Scotland, Canada, the three major coasts of the United States, Puerto Rico, Bimini, Japan, Korea, Vietnam, and Australia) have been studied. From distant points live snails were mailed to us by air, in small quantities of sea water in plastic bags held in stout perforated containers. Snails were maintained in running sea water with bivalves to permit them to bore holes (Fig. 1). Presence or absence, form, and dimensions of the following were associated for each species: bore hole, radula expanded normally over odontophore, salivary glands, accessory salivary glands, pyriform organ, gland and duct of Leiblein, and ABO—structures possibly related to boring. To permit study and drawing of the minute anatomy of these organs, snails were relaxed by means of a Sevin-$CO_2$-freeze technic (Carriker and Blake, 1959). Serial histological sections of ABO and organs of the cephalic hemocoel of most species were prepared to supplement minute anatomical studies.

The ARO is present in all 29 species of boring species examined; in the sole of the foot of 24 Muricidae and Thaididae, and under the distal tip of the proboscis in 5 Naticidae. In *Bedeva hanleyi, Eupleura caudata caudata, E. c. etterae, E. c. sulcidentata, Murex brevifrons, M. cellulosus, M. florifer arenarius, M. fulvescens, M. pomum, Muricopsis ostrearum, Ocenebra erinaceus, O. japonica, Pterorytis foliata, Thais deltoidea, T. emarginata, T. lamellosa, T. lapillus, T. rustica, Urosalpinx cinerea cinerea, U. c. follyensis, U. perrugata,* and *U. tampaensis,* the ABO appears as a separate organ in the anterior mid-ventral portion of the foot, anterior to the egg capsule gland in the female—except in those of the species of *Bedeva, Murex,* and *Muricopsis* listed here which lacked an egg capsule gland

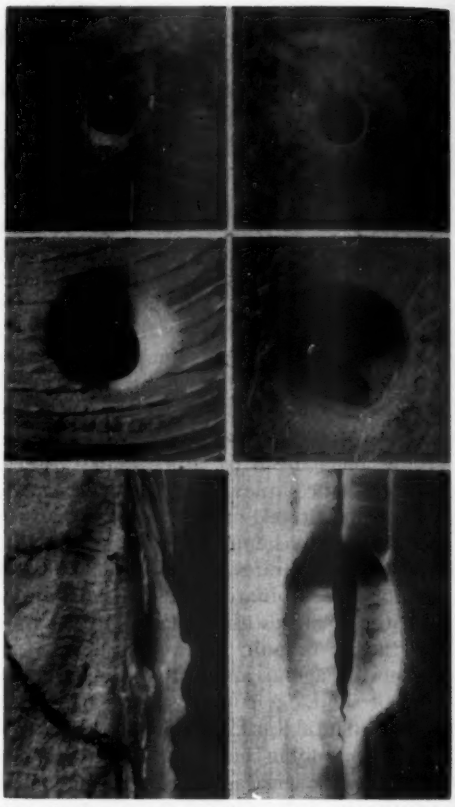

FIG. 1. Some representative gastropod bore holes: upper left, bore hole drilled by *Ocenebra erinacea* (from England) in shell of *Crassostrea virginica*; upper right, by *Bedeva hanleyi* (Australia) in *C. virginica*; middle left, by *Natica severa* (Korea) in *Mercenaria mercenaria*; middle right, by *Murex brevifrons* (Puerto Rico) in *C. virginica*; lower left, by *Thais haemastoma floridana* (North Carolina) in *C. virginica*; lower right, by *Murex fulvescens* (North Carolina) in *M. mercenaria*. 5 ×. (Photographs by S. M. Boone and M. R. Carriker.)

when examined. In *Thais clavigera, Rapana thomasiana, Thais haemastoma, T. h. floridana,* and *T. h. haysae,* the ABO lies atop, and is continuous with, the egg capsule gland, so that in eversion the ABO passes through the cavity of the egg capsule gland; these snails are all effective borers. Two small arteries and two nerves pass to the ABO from the cephalic hemocoel. Absence of accessory salivary glands in *Murex pomum* and their extremely small size in *Bedeva hanleyi, M. fulvescens,* and *M. f. arenarius* depreciates the possible role of these glands in the boring process—as does the fact, reported by Graham (1941), that secretion of the accessory salivary glands of *T. lapillus* has a pH of 6 and does not etch shell or dissolve shell flakes. In Naticidae (*Lunatia lewisi, Natica severa, Neverita didyma, Polinices duplicatus, Sinum perspectivum*) accessory salivary glands are likewise lacking. Histologically the distal cap of the ABO in all borers consists of a very tall, ciliated, glandular epithelium different from any other tissue in these snails. Non-boring predatory snails, like *Buccinum undatum* and *Neptunea decemcostata,* lack an ABO and accessory salivary glands, and their proboscides and radulae are equipped for tearing and pulling flesh rather than for rasping shell.

The proboscis was amputated from each of 16 adult *Urosalpinx cinerea* and 16 adult *Eupleura c. etterae* (equal number of both sexes), and the ABO from a similar sample. Patients were housed with oysters in separate containers in running sand-filtered sea water. All snails survived. In proboscisectomized snails, resumption of drilling in different individuals varied from the 11th to the 34th day after excision; in ABO-ectomized snails, from the 8th to the 25th day. In every case drilling snails possessed fully formed, though small, radulae and ABOs. Length of radula at time of resumption of boring ranged from 1.5 to 5.0mm in snails whose radulae normally ranged from 7 to 11 mm in length. Of the 64 excised snails, only one large, ABO-ectomized female *U. cinerea* did not resume drilling: when dissected after 67 days she had not regenerated the ABO. Other studies on rate of regeneration of the ABO and proboscis showed that no snail resumed boring until both these organs had achieved at least miniature forms of those in normal animals.

By means of the Prytherch viewer (a live shucked oyster held in a cupped oyster valve sandwiched between two glass slides in sea water) we demonstrated repeatedly that *Urosalpinx* and *Eupleura* alternate use of ABO and radula during boring. In early stages of perforation the snail spends most of its time rasping periostracum from the shell surface and exploring the boring site with propodium and proboscis tip. After active boring begins the snail rasps steadily at the boring site for a few minutes, then retracts the proboscis bringing the ABO directly over the hole, extends the ABO into the hole, and leaves it there, tightly sealed from outside seawater by the ventral surface of the foot for periods varying from a few to 55 minutes; it then withdraws the ABO and continues rasping.

Secretion of the ABO, whether from the retracted or the extended and functioning gland, is neutral. ABOs excised from several individuals of 16 of the species of borers listed above and placed on smooth shell in a moist chamber (Ankel, 1937) produced marks on the shell surface ranging from faint etchings to slight depressions the shape and size of the gland. Even though the glands were placed on shell in a drop of sea water two to three times the diameter of the ABO, etchings occurred only under the gland, often leaving sharp boundaries between etched and unaffected shell surface. No erosion took place under control tissues from other parts of the body. Optical and electron micrographs of metal-shadowed collodion and collodion-carbon replicas and pseudo-replicas of these etchings show that the secretion softens and loosens the surface crystals of shell calcite; it also etches the surface of non-biological inorganically deposited calcite and aragonite crystals. Action of the secretion on the conchiolin (freed of calcite with disodium ethylene diamene tetraacetate) of the shell, and nature of the secretion are under study.

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# NEUROHORMONES OF MOLLUSCA

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We may define neurohormones as substances of high physiological activity that are produced by nerve cells and released at their axon terminations to act as short-range, brief-duration transmitter agents, or as long-range, long-duration circulatory hormones. The short-range transmitters may be referred to as neurohumors, and the more characteristic hormones as neurosecretory substances. I shall summarize, briefly, our knowledge of neurosecretion in molluscs, then deal with the neurohumors and, especially, with 5-hydroxytryptamine or serotonin, which has attracted so much attention during the past few years.

## NEUROSECRETION

Scharrer (1935) was the first to describe neurosecretory cells in molluscs. In fact, her distinguished career in the field of neurosecretion was launched with her study of the spectacularly large neurosecretory cells of the opisthobranchs, *Aplysia* and *Pleurobranchaea*. One of the earliest observations of the occurrence of neurosecretory substances in axons was also made by Scharrer on *Pleurobranchaea*. It is now generally recognized that neurosecretory substances are formed in the cell body of a neuron and are carried by axoplasmic flow to the terminals for storage and release.

In a series of papers, Gabe (1949, 1951, 1953a and b, 1954, 1955) reported finding neurosecretory cells in a wide variety of molluscs, including a scaphopod, some 25 species of prosobranchs, 35 species of opisthobranchs, and representatives of four orders of lamellibranchs. No one, to my knowledge, has yet found neurosecretory cells in amphineura or cephalopods, and, thus far, a properly fixed specimen of the monoplacophoran, *Neopilina*, has not fallen into the hands of a neurosecretion enthusiast.

In the insects and crustaceans, several types of neurosecretory cells may be found in a given species. Lever, (1957, 1958a and b) has described five types of neurosecretory cells in representatives of ancylid pulmonates. More important, perhaps, Lever has found what may be storage-release centers in these molluscs, as well as an interesting association of special glandular areas with neurosecretory cells of the cerebral ganglia. He suggests that these may be analogous with the brain-corpus cardiacum system of insects, the X-organ-sinus glands of crustaceans, and the hypothalamic-pituitary neurosecretory system of vertebrates.

In this country, studies on neurosecretory systems in molluscs have only recently begun. Durand (personal communication) has made a study of neurosecretory cells in *Venus mercenaria*, and has found cells in the cerebro-pleural ganglia and in visceral ganglia containing granules that stain with certain of the selective neurosecretory stains. Gabe (1955) reports finding no neurosecretory cells in the pedal ganglia of lamellibranchs but Durand has seen what he believes to be neurosecretory cells in pedal ganglia of *Venus*.

Loveland (personal communication) has made a preliminary electron microscope study of *Venus* ganglia. He finds occasional axons filled with electron-dense granules with characteristics similar to those seen in neurosecretory neurons of arthropods and vertebrates. The infrequency with which such granule-filled axons are seen corresponds with the relatively small numbers of neurosecretory cells compared with ordinary neurons in these ganglia.

Much remains to be done on neurosecretion in molluscs. The important questions of the chemical nature and diversity of neurosecretory substances in this group, and their physiological roles, are yet to be answered. The observations of Gabe, as well as those made recently by certain workers in Belgium (Herlant-Meewis and Van Mol, 1959) indicate that cycles of secretory ac-

tivity of some neurosecretory cells in molluscs are related to the reproductive cycle. Since the reproductive cycle is usually seasonal and, in turn, related to environmental factors of temperature, food abundance and so forth, it is not entirely safe to conclude that a neurosecretory substance regulates the activity of the gonads; instead, it is possible that both gonads and neurosecretory cells are under the same environmental influences.

## NEUROHUMORS

A neurohumoral role for acetylcholine (ACh) in molluscs is now certain. The actions of some cardio-regulator neurons are mediated by this agent. Early evidence came from the work of Prosser (1940) on the heart of *Venus mercenaria*. A variation of the Prosser experiments, using an isolated heart is seen in Fig. 1. Firstly, this record shows that an isolated *Venus* heart can be inhibited by direct electrical stimulation and that it recovers soon after the stimulus is stopped. Secondly, it shows that the duration of inhibition is greatly prolonged after the heart has been treated with an anti-cholinesterase; in this case,

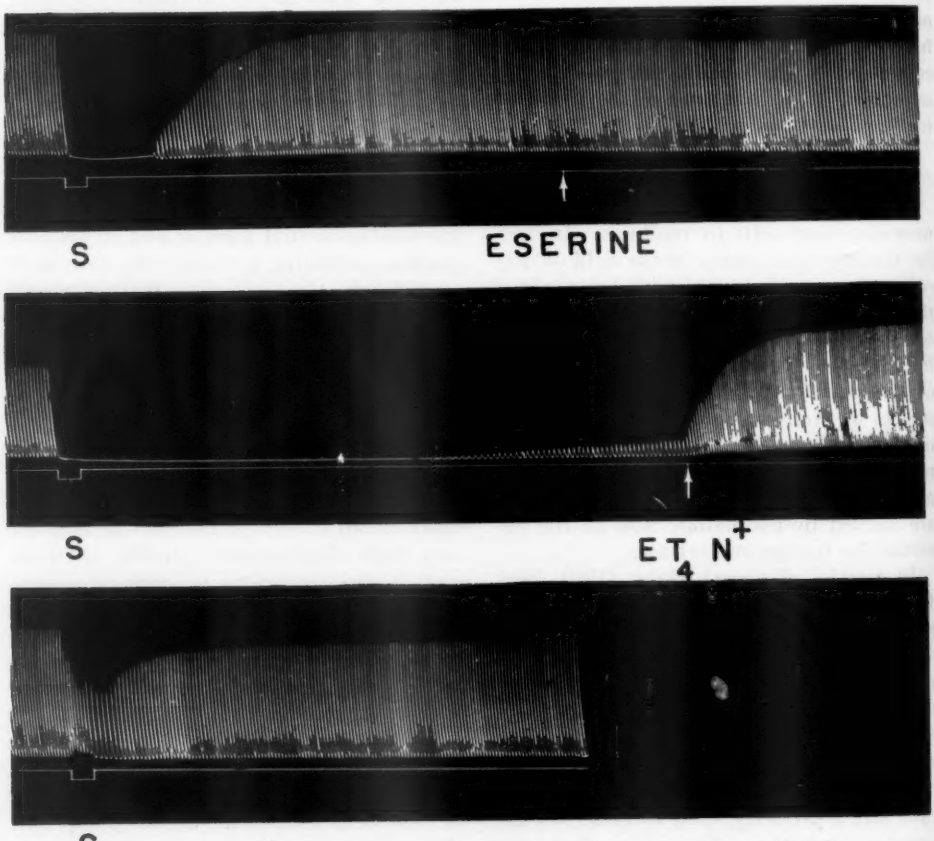


FIG. 1. A demonstration of cholinergic, inhibitory regulation of the heart of *Venus mercenaria*.

Upper trace: Isolated heart stimulated at S for 30 seconds with repetitive electrical stimuli. At the arrow, eserine was added to the bath to a final concentration of $10^{-5}$ g/ml.

Middle trace: At S, electrical stimulation repeated as above. At arrow, tetraethylammonium chloride was added to the bath to a final concentration of $10^{-4}$ g/ml.

Lower trace: At S, electrical stimulation repeated as in two upper traces.

TABLE 1. *Relative activities on* Venus *heart of acetylcholine analogues prepared from pentanones* (from Welsh & Taub, 1951)

| Compound | Structural formula | Equiactive molar ratios, mean ± SE |
|---|---|---|
| Acetylcholine bromide | $[(CH_3)_3N^+CH_2CH_2OCCH_3]\,Br^-$ (O) | 1 |
| 4-Ketoamyltrimethylammonium iodide | $[(CH_3)_3N^+CH_2CH_2CH_2CCH_3]\,I^-$ (O) | 12 ± 0.9 |
| 3-Ketoamyltrimethylammonium iodide | $[(CH_3)_3N^+CH_2CH_2CCH_2CH_3]\,I^-$ (O) | 160 ± 36 |
| 2-Ketoamyltrimethylammonium iodide | $[(CH_3)_3N^+CH_2CCH_2CH_2CH_3]\,I^-$ (O) | 620 ± 56 |

eserine. Thirdly, inhibition is quickly abolished when the heart is bathed by an appropriate acetylcholine antagonist, such as tetraethylammonium. Mytolon would have been even more effective, but it had not been synthesized when this record was made.

We now know a great deal about the pharmacology of the *Venus* heart and some significant, basic observations have been made with this preparation. I mention only one. In the reaction of acetylcholine with receptor substance, the importance of the cationic head of the acetylcholine molecule was early recognized. The full significance of the carbonyl group, however, was not clear. A series of ketone derivatives resembling ACh but having the carbonyl group in three different positions was made (Welsh and Taub, 1951). Table 1 shows their relative activities on the *Venus* heart. Clearly, the compound with its carbonyl group in the same relative position to that in ACh is the most active. This, and other evidence, such as the loss of activity with reduction of the ketone oxygen, points clearly to the importance of the carbonyl group in the reaction of ACh with its receptor sites.

So, molluscan hearts respond to ACh—not always by being inhibited, however. Hearts of all members of the genus *Mytilus* thus far examined are excited by ACh. Molluscan body muscle, such as that of the radular apparatus (Hill, 1958), and the anterior retractor of the byssus of *Mytilus* (Twarog, 1954) is excited by ACh. Ciliary beat appears to be under the control of the ACh system. In addition, molluscan nervous systems contain ACh and cholinesterase. We may conclude that ACh serves an important transmitter role in molluscs, as it does in most groups of animals.

There is good evidence that much molluscan muscle, both body muscle and cardiac muscle, is doubly innervated. Furthermore, there is central excitation and inhibition and, in *Aplysia*, ACh appears to be the transmitter at certain central inhibitor synapses (Tauc and Gerschenfeld, 1960). If ACh is involved as a mediator of one set of neurons—either excitor or inhibitor, depending on the specific junction—what is the nature of the opposing mediator? The catecholamines, adrenaline and noradrenaline, have naturally been thought of, but their actions are generally weak in molluscs and do not closely parallel those produced by nerve stimulation. The first clues suggesting a new type of mediator or neurohumor came from the observations of Erspamer and Ghiretti (1951) that enteramine, a product of enterochromaffin cells, had a remarkable excitor action on certain molluscan hearts. Within a year, enteramine had been shown to be 5-hydroxytryptamine (5-HT, serotonin) and synthetic material was found to have an action identical with that of enteramine on the octopus heart by Bacq, Fisher, and Ghiretti (1952). We had sometimes seen excitation of the *Venus* heart following inhibition produced by electrical stimulation of the visceral ganglion. After an ACh-blocking agent was

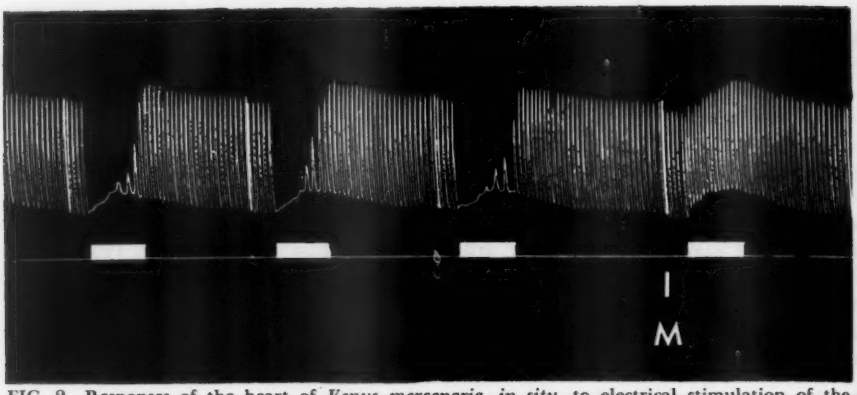


FIG. 2. Responses of the heart of *Venus mercenaria, in situ,* to electrical stimulation of the visceral ganglion. After three periods of stimulation, a few drops of $10^{-5}$ g/ml of Mytolon were applied to the heart at M. After a delay of 5 min the ganglion was again stimulated as before.

applied to the heart, only excitation was seen (Fig. 2). When synthetic 5-HT became available in 1952 we tried it on the *Venus* heart and found that it excited the heart in low concentrations and in a way that closely paralleled that produced by nerve stimulation after ACh blockade (Welsh, 1953). Following this, it was important to know whether 5-HT was to be found in the molluscan nervous system. We began to look for it in *Venus* ganglia, which, by chance, turned out to be highly favorable material. By multiple bioassay, paper chromatography, and characteristic fluorescence, we were able to show that 5-HT is present in *Venus* and other molluscan nervous systems (Welsh and Moorhead, 1959; 1960). Some of the data from these papers is summarized in Table 2.

Brief mention of some of our own work that is now in progress and of some recently published work of former students, and of others, follows. Reserpine, one of the *Rauwolfia* alkaloids and a well known tranquillizing agent, causes the release of stored 5-HT from the mammalian nervous system. It has a similar action in molluscs. As little as 10 $\mu$g of reserpine injected into a large specimen of the Florida gastropod, *Melongena coronata,* induces a kind of sedation or tranquillization that persists for longer than three weeks. Fig. 3 shows the difference in behavior of normal and reserpinized *Melongena.* At the time of full sedation, the 5-HT of *Melongena* ganglia is about one fifth of the normal level (Welsh and Mirolli, unpublished).

TABLE 2. *5-HT Content of some molluscan ganglia* (from Welsh and Moorhead, 1960)

| Class | No. of species | $\mu$g 5-HT/g tissue Range | Mean |
|---|---|---|---|
| Pelecypoda | 7 | 12 -40 | 25 |
| Gastropoda | 6 | 2.5- 9 | 6.5 |
| Cephalopoda | 4 | 0.9- 3.2 | 2.2 |

We continue to work with the effects of certain of the remarkably active derivatives of lysergic acid on the *Venus* heart. One of these, d-lysergic acid diethylamide or LSD, in minute amounts, causes in a normal human a state resembling schizophrenia. On the *Venus* heart, LSD, in trace amounts, causes an excitation resembling that produced by 5-HT. As little as $10^{-16}$ M LSD will produce, in time, a maximal increase in amplitude of beat. This action is only partially reversed after prolonged washing. Some closely related lysergic acid derivatives such as 2-bromo- LSD have little or no excitor action and instead are effective 5-HT antagonists (Welsh and McCoy, 1957).

Twarog continues to study the anterior retractor byssus muscle of *Mytilus.* This muscle can be made to contract tonically by a cathodal DC pulse and by ACh. It can be made to relax by a brief burst of

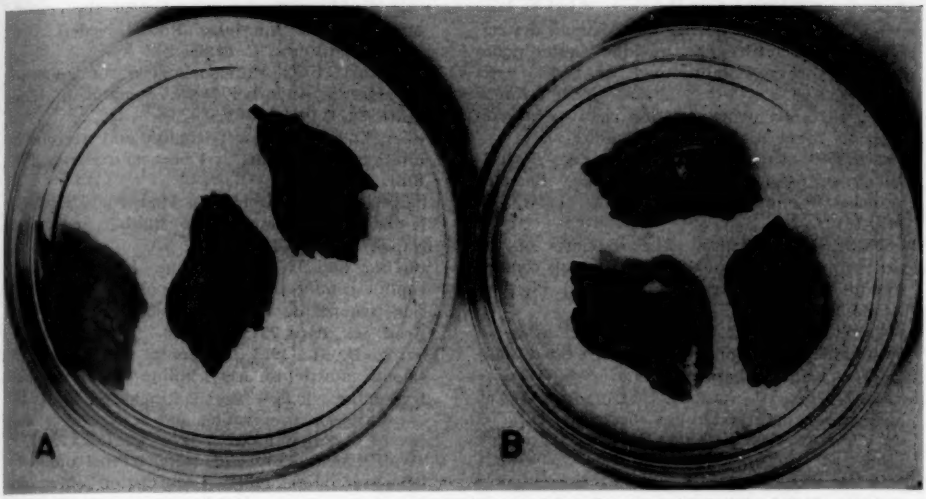


FIG. 3. Response of *Melongena coronata* to reserpine. A. Controls. B. Animals injected one week earlier with 100 μg of reserpine phosphate. Such animals are unable to extend the foot and right themselves although response times to prodding the foot are essentially unchanged.

anodal stimuli and by 5-HT. It would appear that the proper electrical stimulation may selectively release, or mimic the actions of, ACh and 5-HT in this muscle (Twarog, 1960a and b).

Greenberg (1960a and b) has recently published two outstanding papers on molecular structure and biological activity of amines and tryptamine analogs on the heart of *Venus mercenaria*. He has done much to further our knowledge of the parts of the 5-HT molecule that are most significant in its reaction with receptor substance. By selecting a series of compounds, Greenberg has shown that the indole nitrogen, the 5-hydroxyl group, and the amino group all contribute to the specificity of action of 5-HT.

Work on 5-HT and ACh actions in molluscs goes on in Europe. For example, Meng (1960) has done a recent study of their actions in the *Helix* heart and Kahr (1959) has shown them to have opposing actions on the chromatophores of *Octopus vulgaris*. Mention of much other work must be omitted for lack of space.

## SUMMARY

In this brief consideration of neurohormones in molluscs many significant studies have not been mentioned. However, from the examples cited, it is clear that molluscs possess neurosecretory systems that are comparable with those found in arthropods and vertebrates, although less is known concerning their physiological roles.

Our knowledge of the nature and action of the neurohumors, such as acetylcholine and 5-hydroxytryptamine, in molluscs, is advancing rapidly. Much work remains ahead and, as experimental animals, the molluscs hold the promise of rich rewards to those interested in invertebrate neuroendocrines.

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# PHYSIOLOGY OF REPRODUCTION IN MOLLUSCS

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The present review deals with three major aspects of reproduction in molluscs: the anatomy of gonads and secondary sex organs; the reactions involved in the discharge and fertilization of the sex cells; hermaphroditism and sex change.

## ANATOMY

In the primitive type of reproductive system found in *Amphineura,* in many bivalves, and in certain archaic Prosobranch gastropods, the germ cells are shed directly into the surrounding water and the fertilization of eggs takes place outside the maternal organism. A more elaborate system for the transmission of spermatozoa by means of copulatory organs has developed to various degrees of complexity in gastropods. Essentially this system is formed by the extension and differentiation of the male genital opening accompanied by the development of various accessory glands on the walls of the genital glands. In the cephalopods, sperm, enclosed in highly specialized spermatophores, are transmitted to the female by the modified arms. In some species the latter organs detach themselves from the animal and attain a certain degree of independence which permits them to swim over a considerable distance.

In spite of great variation one primitive characteristic is common to all the molluscs, namely, that the inner cavity of the gonads corresponds to the coelom and the sex products are discharged into the pallial cavity through the gonoducts which in many species retain connection with the nephridia.

The evolution of the reproductive organs from a most simple gonad found in bivalves, devoid of any accessory organs, to the most complicated system of pulmonates and cephalopods follows different patterns which serve to increase the chances for fertilization of eggs, to make possible correct timing of spawning and selective mating by various tropismatic reactions, and to promote survival of the progeny by various means of protection of developing eggs. By these methods a high level of population density may be maintained.

The gonad of the bivalves is a paired structure which in some species is fused in the middle. Its body consists of an acinous gland with many ramified blind tubules which open into ciliated canals. The latter join into a gonoduct through which the sex cells are discharged into the pallial cavity (Fig. 1). These ducts are usually short and ciliated, and they have no glands. In *Nucula* and some other genera, the sex gland opens into the nephridia and the sex cells are discharged through a common genitorenal duct.

The gonad may be located at the base of the visceral mass or in front of it, as for instance in *Neotrigonia.* More often, however, the sex glands extend into the mantle (*Mytilus, Yoldia*) and into the surface of the visceral mass, in which they form a ring-like layer, as for instance in oysters, clams, and pearl oysters (*Pinctada* sp.). In some bivalves marked pigmentation distinguishes the ovaries from the testes (sea scallop) while in others there are no such differences between the sexes. In hermaphroditic *Pandora,* ovaries occupy a dorsal position and are clearly separated from the more ventrally located spermaries. Such a topographical difference is not found, however, in the hermaphroditic species of oysters (*O. edulis, lurida*) in which eggs and sperm may develop in any part of the gonad.

The bulk of the gonad greatly varies with the season. The fully developed gonad of *C. virginica* forms a layer several millimeters in thickness, and in exceptional cases it may exceed 10-12 mm. The highest gonad development is reached shortly before

spawning. At such a stage the ovary or the testis of *C. virginica* may form over 40% of the total body volume and weight, exclusive of shell. After spawning and absorption of the remaining ovocytes by phagocytes, the gonad is reduced to a few narrow islets of germinal epithelium. At this indifferent stage the sex of the mollusc is not recognizable.

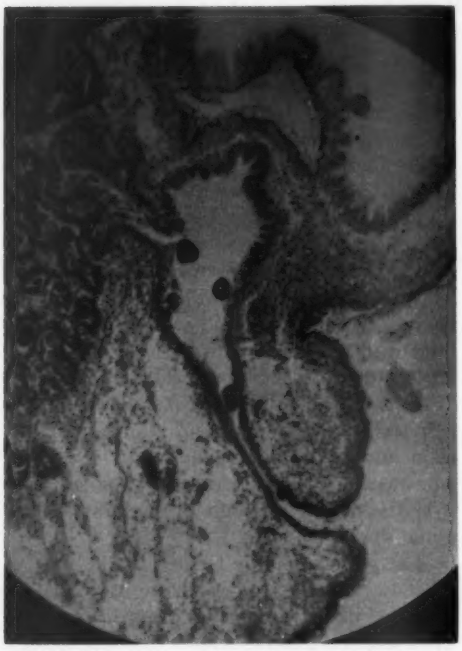

FIG. 1. Transverse section of an ovary of sexually mature *Crassostrea virginica* preserved during ovulation. Eggs are passing through a short gonoduct. Kidney reservoir on the top. × 50.

### DISCHARGE OF GAMETES AND FERTILIZATION

Accessory sex organs are absent in the bivalves. The dispersal of eggs by the oviparous species, or of larvae by larviparous forms, is accomplished by the coordinated action of the adductor muscle, the epithelium of the gills, and the edge of the mantle.

In the majority of bivalve species the eggs are fertilized outside of the maternal organism and are left to their own fate. In fresh water *Unionidae* and some marine species, the eggs are incubated inside the female. In the simplest form this parental care of the progeny is accomplished by retaining the eggs inside the gills between the gill lamellae (*Ostrea edulis, O. lurida*). In fresh water *Unionidae* (Fig. 2) the gill tissue is modified to form brood chambers of various degrees of complexity. In *Sphaerium* the cells of the chambers supply glycogen, which is utilized by the embryos (Couteaux-Bargeton, 1948).

The oviparous molluscs as a rule produce an enormous number of eggs, the majority

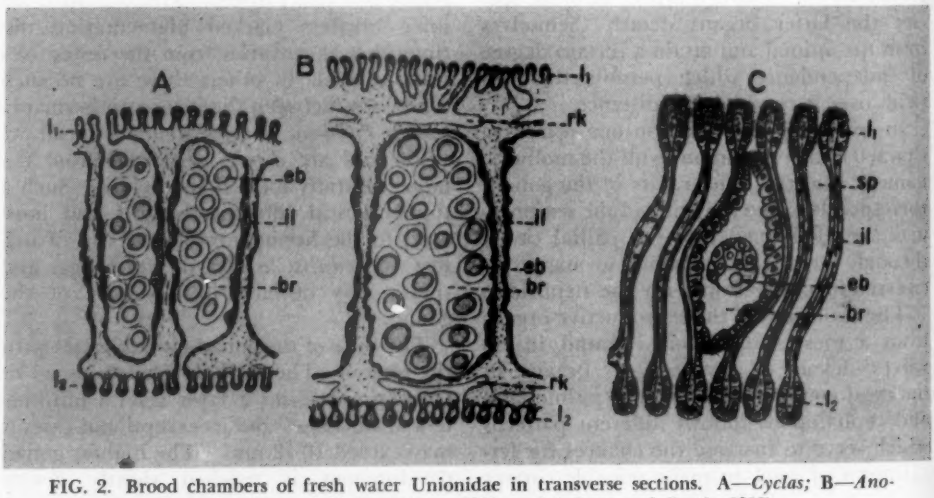


FIG. 2. Brood chambers of fresh water Unionidae in transverse sections. A—*Cyclas;* B—*Anodonta;* C—*Quadrula.* (From J. Meisenheimer, 1921; and Lefevre and Curtis, 1912).

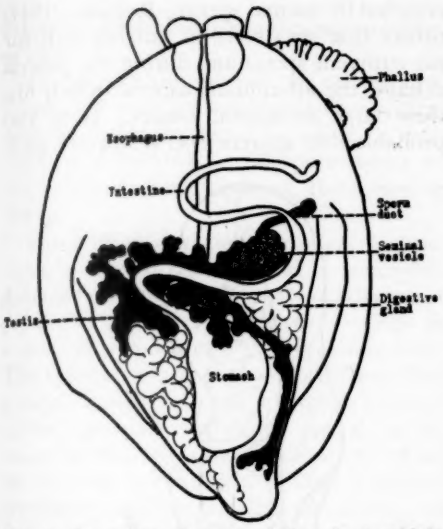


FIG. 3. Sex organs of small adult male *Crepidula plana*. (From Fig. 13, H. N. Gould, 1952, J. Exp. Zool., 119:131, with permission.)

of which perish before completing larval development. In other molluscs the apparent tendency for the economy of gametes is accomplished in various ways: by the elimination or shortening of the pelagic life of the larvae, as for example in *Macoma* and *Nucula* which lay only a few eggs; or by the failure to shed the sex products unless close to one of the other sex, as in the gastropod *Patella*, in some chitons, and in *Haliotis*.

Further evolution in the structure of organs of reproduction results in the appearance of special genital ducts and copulatory organs which convey, receive, and store the sperm for internal fertilization; in the development of the glands which secrete protective membranes for eggs; and in the development of special accessory organs used for sexual stimulation in mating.

A special organ of copulation, the phallus, of gastropods is usually a conspicuous organ noticeable without dissection. The development of the phallus is accompanied by the appearance of the seminal vesicle for the storage of sperm and the spermiduct, as can be seen, for instance, in the anatomy of *Crepidula plana* (Fig. 3). The sexual dimorphism is frequently, but not always, expressed in the difference in size—the males being considerably smaller than the females (Fig. 4).

The greatest complexity of reproductive system is attained in pulmonates. The organs of reproduction of these snails consist of the following main parts (Fig. 5): the hermaphroditic gland or ovo-testis (*o.t.*) and its duct (*h.d.*); the uterine enlargement of the duct (*u.*); the receptacle for storage of sperm which opens into the female gland (*r.s.*); albuminous gland (*alb.g.*) which secretes the egg membranes; finger-shaped accessory gland (*a.c.g.*); calciferous gland and sac full of calcite darts (*d.s.*) or arrows used in sexual stimulation. The male counterpart of the apparatus also begins with the ovo-testis from which the sperm is discharged through the spermiduct or vas deferens (*v.d.*); it has a phallus (*p*) with a long, hollow caecum or flagellum which secretes the spermatophores used for the transmittal of semen. In some gastropods the sper-

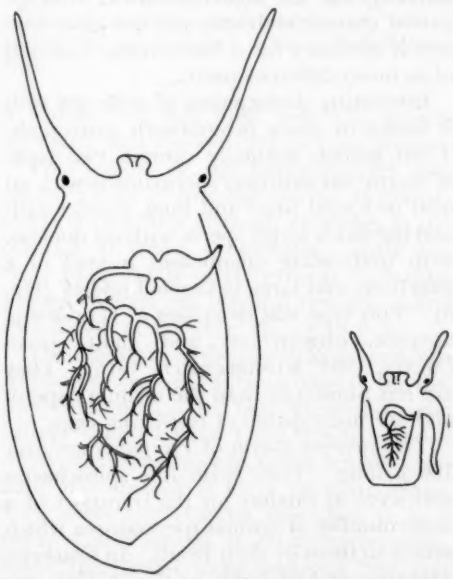

FIG. 4. Two successive stages in the development and absorption of phallus in *Crepidula nummaria*, from the mated male (right) to female phase (left). The phallus is absorbed and the uterus and ovary developed instead of seminal vesicle and spermary of the male. Adapted from W. R. Coe (1938).

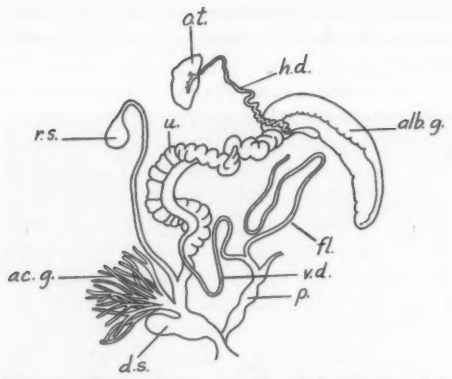


FIG. 5. Reproductive system of hermaphroditic land snail *Helix pomatia,* adapted from J. Meisenheimer (1921). ac.g.—finger-shaped accessory gland; alb.g.—albuminous gland; d.s.—calciferous gland and sac of calcite darts; fl.—flagellum; h.d.—duct from ovo-testis which leads to the enlarged oviduct and vagina (u.), and spermiduct; p—phallus; r.s.—seminal receptacle and its duct; v.d.—spermiduct.

matophores are furnished with denticulations, hooks, and similar structures (*Nania wallacei*) and are highly complex. It is apparent that an elaborate physiological control is necessary for a harmonious function of so many different parts.

Interesting dimorphism of male sex cells is found in many prosobranch gastropods. Their gonads normally contain two types of sperm: the ordinary spermatozoa with an oval or round head and long, slender tail; and the much larger sperm without nucleus, with undulating membranes instead of a flagellum, and large hexagonal bodies (Fig. 6). This type was described as vermiform, apyrene, oligopyrene, and giant sperm (Meves, 1903; Kushakewitch, 1913). They are less numerous than the ordinary sperm and are not capable of fertilizing eggs.

The apyrene sperm of *Viviparus* are over 100 $\mu$ long. They swim like spirochaetes and serve as carriers for the transport of a large number of normal spermatozoa which attach to them by their heads. In *Clathrus, Janthina,* and probably in *Cerithiopsis,* the apyrene spermatozoa are used for active delivery of normal sperm into the oviduct. In *Strombus bituberculatus* after copulation they are found only in the uterus but never in the seminal receptacles which are crowded by normal sperm. Reincke (1914) thinks that they serve as nursing cells for the eupyrene sperm and during this process exhaust the albuminal material which fills their large hexagonal bodies. It is also probable that apyrene sperm liberate some

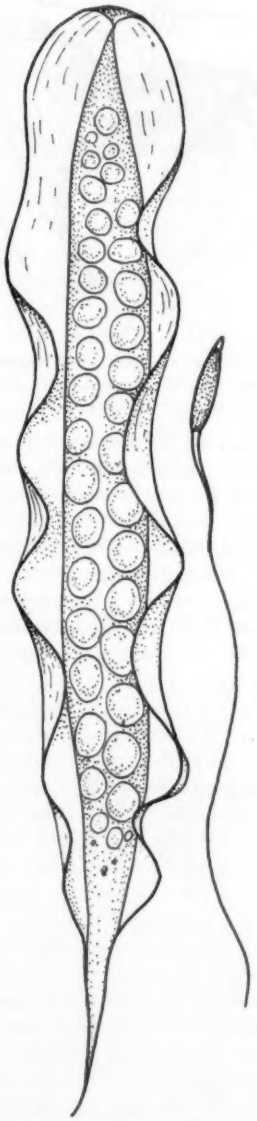

FIG. 6. Apyrene (left) and eupyrene (right) spermatozoa from the uterus of *Strombus bituberculatous.* From E. E. Reinke (1914).

substance which facilitates the insemination of eggs.

The dimorphism of male sex cells in Prosobranchia may be considered as a special adaptation for successful breeding of molluscs by delivering fertile spermatozoa to the uterus and maintaining them during storage.

The evolution of the reproductive apparatus of cephalopods follows a somewhat different direction. The male organs are of particular interest. The testis develops as a specialized portion of the coelomic wall. The spermatozoa pass through the so-called genital capsule into the spermiduct which opens externally into the pallial cavity either on the right side as in *Nautilus* or on the left side in *Dibranchia*. One or several glandular pouches on the spermiduct of *Dibranchia* show further specialization. They develop into the vesicula seminales and the prostate and form the terminal reservoir or Needham's sac for the storage of spermatophores. The sperm entering the initial part of the spermiduct is free; in the first glandular pouch it becomes surrounded by an envelope which is an invaginated tube, the deeper part of which constitutes a storage reservoir, and the more external part of which is greatly contracted and coiled into a spiral. In many species the spermatophores are small (Racovitza, 1894a, 1894b) but in *Eledone* they attain the length of 8 cm and in some Octopoda they are much longer. The longest ones appear to belong to the Pacific species, *Octopus honkongensis*; some of them when uncoiled were found to be almost four feet long (Lane, 1960).

The organ of copulation reaches a high degree of development in *Nautilus*. It is made of four modified tentacles, usually on the left side of the body. The arms are united to form a projection surrounded by a glandular area. This structure is called the spadix. In *Loligo, Sepia, Rossia,* and others, one arm is modified into a copulatory organ, the hectocotylus, which in some Octopoda (Fig. 7) becomes autonomous; when detached it is able to live and move until it penetrates into the pallial cavity of a female, attaches itself near the genital aperture, and releases the spermatophores.

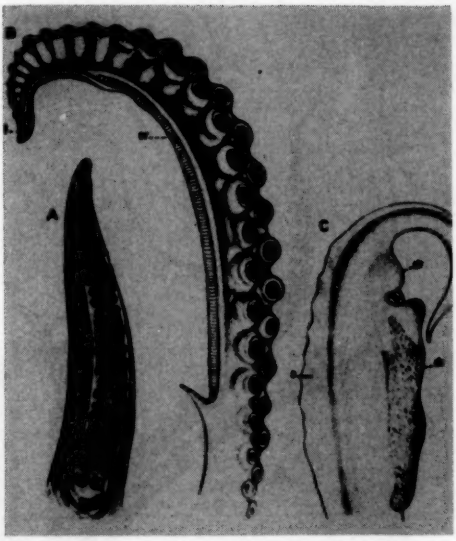


FIG. 7. Hectocotylus arms of three species of cephalopods. A—*Sepia orbignana;* B—*Octopus defilippi;* C—*Pterygoteuthis giardi* according to Jatta, 1896 (A and B) and Chun, 1910 (C). dr.—glandular cushion; l.—spoon-like tip; s.—swimming border; s.r.—spermatophore groove. From J. Meisenheimer, 1921.

The length of the hectocotylized arm greatly varies in different species. It reaches the very large size of about 16 inches in the octopus *Ocythoe tuberculata,* while the body of the male rarely exceeds 10 inches. Before detachment the arm remains coiled in a sheath or sac (Fig. 8).

The discovery of the male paper nautilus, *Argonauta argo,* is a famous page in the history of zoology. In 1827 Stephano delle Chiaje (quoted from Lane, 1960) found a small organism attached to the female argonaut and mistook it for a parasitic worm. Two years later Cuvier described it as a parasite and named it *Hectocotylus octopodis,* the generic name meaning "the arm of a hundred suckers." The error persisted until 1853, when Heinrich Müller published the first correct description of the male. The term hectocotylus survived and denotes now the modified arm of a male cephalopod.

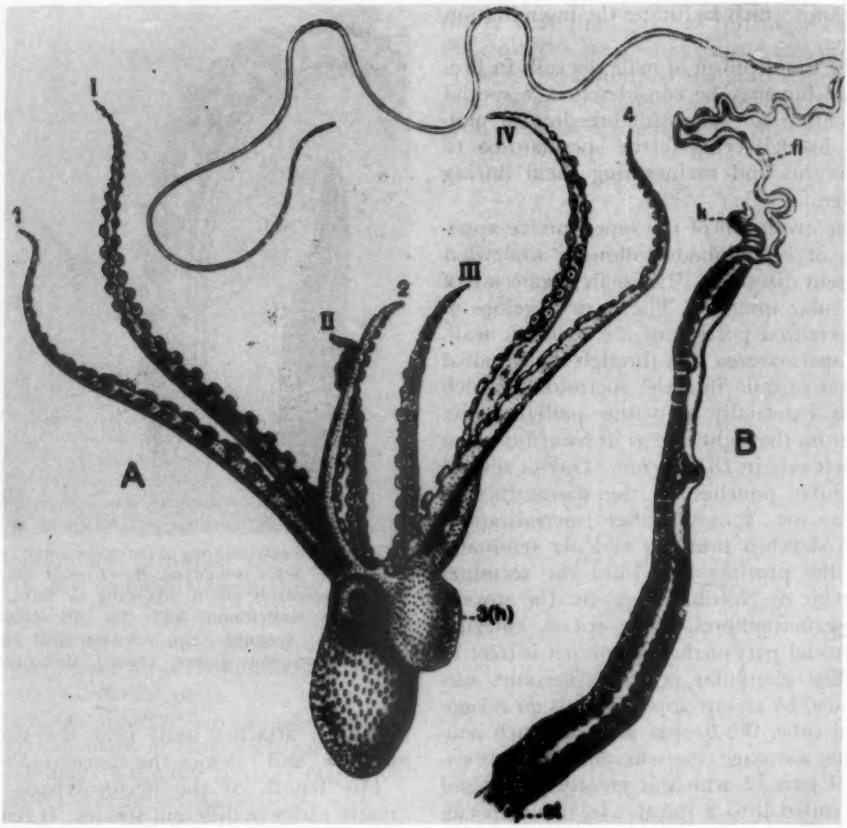


FIG. 8. Side view of the male *Ocythoe tuberculata* (A); (B)—detached and uncoiled hectocotylus arm. fl.—flagellum; k.—capsule of the arm's tip; st.—stalk of the hectocotylus; I-IV—the four arms of the left side of the body; 1-4—the arms of the right side. According to G. Jatta, 1896. From J. Meisenheimer, 1921.

The view found in zoological literature that the male argonaut is a parasite on a female body is not substantiated by observations. Males of this species were collected in plankton hauls and apparently are capable of living independently of females.

## SEXUAL ACTIVITIES

The sexual rhythm is a cyclic phenomenon which starts with the development and maturation of sex cells and ends with their discharge. It may be of short duration repeating itself every month, as for instance in the tropical marine bivalves, or it may be seasonal and coincide with the rise of temperature to the optimum level typical for a given species (Orton, 1920; Coe, 1933, 1936a, 1936b; Korringa, 1957; Giese, 1959). A good example of such seasonal periodicity may be found in the American oyster, *Crassostrea virginica,* from the North Atlantic states. The proliferation of sex cells starts in this oyster with the onset of warm weather early in April and reaches the climax in the warmest months of the season, July and August. In the males the act of spawning begins with the liberation of sex cells from the follicles. Strong ciliary action propels the sperm through the genital ducts into the epibranchial chamber and on to the outside. No other organs are involved

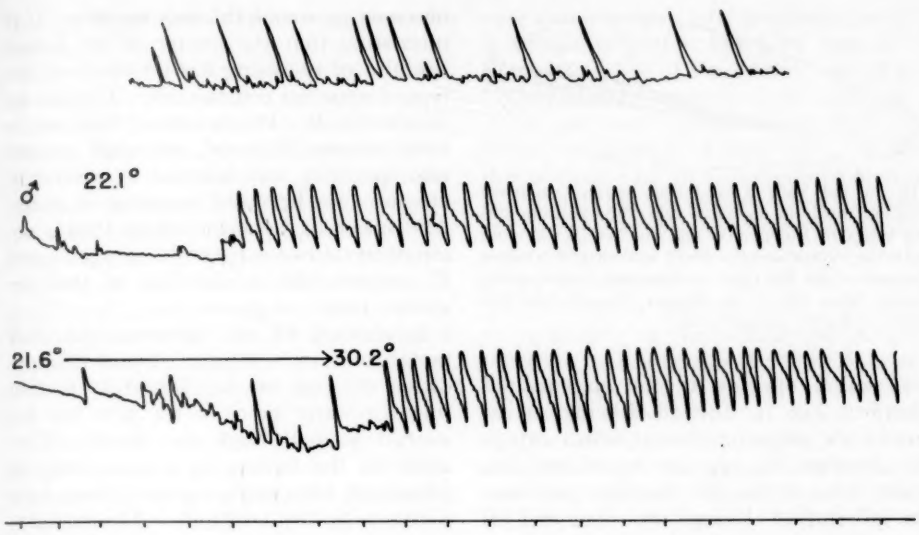


FIG. 9. Kymograph tracings of shell movements of *Crassostrea virginica* from Buzzards Bay, Massachusetts. Upper line—male oyster; middle line—sexually mature female stimulated to spawn by the sperm (♂); lower line—another sexually mature female stimulated to spawn by rapid rise of temperature from 21.6° to 30.2° C. The emission of sex cells was very copious in all three oysters.

in this simplest form of male spawning act. The release of mature spermatozoa can be triggered by a sudden rise in temperature or by various chemical substances, some of which may be normally present in the water while the others never occur in natural environment. The spawning of the male is provoked by the eggs or egg water of the same species, by the eggs and blood of other bivalves, and by thyroidin, various sugars, egg albumen, and extracts of sea weeds (Galtsoff, 1930, 1940). A ripe male oyster responds to these substances within a few seconds after their application to the mantle edge and gills. At the height of the spawning season the shedding of the sperm, once started, may continue for hours until the male is almost completely spent. Frequently, however, the shedding of sperm induced by chemical stimulus lasts a short time, from a few seconds to several minutes, but may be immediately repeated by the additional dose of stimulant. The sperm is dispersed in the surrounding water by the cloacal current without the assistance of shell movements. Occasional contractions of the adductor muscle shown in Fig. 9, first line, were recorded during copious spawning of the male. Sharp snapping of the valves are ejection reactions typical for normal feeding behavior of the oyster in water containing a large quantity of suspended particles. These contractions are not associated with the emission of sperm.

The response of a sexually mature oyster to chemical stimuli is non-specific. The spawning reaction may be triggered by a great variety of organic substances. It is, therefore, understandable that under natural conditions the spawning of a population of oysters is initiated by the males. This conclusion is confirmed by numerous laboratory experiments and field observations.

The spawning reaction of the female oyster is more complex and involves several organs. The ripe ovary consists of a large number of follicles containing pear-shaped eggs attached to the walls. In spawning the eggs become detached and by strong ciliary action inside the ducts are transported to

FIG. 10. Side view of the female oyster, *Crassostrea virginica* ready to discharge eggs. The pallium and the edges of the mantle seal the pallial chamber with the exception of a small oval-shaped window through which the eggs are dispersed in the water. Drawn from life. The distance between the two valves is slightly exaggerated.

the genital opening in the epibranchial chamber (Fig. 1). Instead of being carried out by the outgoing current which sweeps the chamber, the eggs are forced into the water tubes of the gill lamellae, penetrate the gill surface through the ostia, and fill the pallial cavity. To observe this process in the laboratory it is necessary to remove a part of the valve and stimulate the oyster to spawn. The ciliary motion of the gills is not reversed during spawning; the eggs are forced into the water tubes by the suction caused by the rhythmical contractions of the adductor muscle (Fig. 9, lines 2 and 3). The pallial curtain at this moment is temporarily sealed by the opposite edges of the mantle (Fig. 10) with the exception of a small oval-shaped opening, through which the eggs are dispersed (Galtsoff, 1938). Shell movements during spawning are characterized by the regularity and rapid rhythm of the contractions of the adductor muscle. We have not been able to induce this behavior of the muscle with any of the drugs so far tried.

The passage of eggs through the gills coincides with the moment when the adductor reaches the half-way point in its relaxation phase and the stretching of the muscle is temporarily slowed. This point is clearly marked as a short plateau or ledge on the relaxation curve (See Fig. 9, lines 2 and 3). Upon the completion of the relaxation phase, a sharp snapping of the valves closes the shells and propels the eggs to the outside. A delicate coordination of the functions of the gills, mantle, and adductor is necessary to accomplish this reaction. It is interesting that the nature of the initial stimulus of spawning has no effect on the type of muscular contractions. This can be seen in Fig. 9. The two lower lines are in every respect identical, although in one case spawning was induced by sperm, in another case by rapid warming of water. The spawning behavior which I have observed in *Crassostrea gigas, C. angulata,* and *C. commercialis* is identical to that described for *C. virginica.*

By cutting off the ligament, the shell movements can be stopped. Under this condition the eggs are not forced by suction into the water tubes of the gills but are washed away through the cloaca. They settle on the bottom in a dense mass in which only a few at the top of the heap have a chance to be fertilized. The complex mechanism for dispersal of eggs developed in the oviparous oysters obviously has value for the survival of the eggs and provides a better chance for fertilization.

Spawning of a sexually mature *C. virginica* may be initiated by a sudden rise of temperature, by sperm of the same or related species, or by the combined action of both stimuli. Sperm of other genera of bivalves are ineffective, and no response can be obtained with various substances which are effective in provoking the shedding of sperm. It is therefore obvious that the sexual response of the female is highly specific.

The latent period of female reaction varies from 6 to 20 minutes (Fig. 9, two lower lines) whereas the male responds to stimulation within a few seconds. Apparently different receptors are involved in the two cases. The long latent period of the female reaction suggests the possibility that the sperm may be absorbed, probably in the intestinal tract or by the gills. It takes about 6 to 8 minutes for a small particle to be delivered by the gills to intestine. The coincidence may be significant, but direct proof is lacking. It is, however, interesting that in the males, which also can be stimulated to spawn by the sperm, the response occurs within a few minutes instead of a few seconds as in the case of stimulation

by egg water and various organic substances.

The active substance which stimulates spawning is extractable from mature spermatozoa by alcohol and benzene (Galtsoff, 1938b). The reaction initiated in the female by such extracts is not different from the effects of live sperm. Nelson and Allison (1940) reported that spermatozoa of the oyster carry another hormone-like substance which causes wide dilation of the ostia and in this way accelerates the transfer of water by the gills. They named the substance diantlin and thought that it was different from the substance which provokes spawning.

Other bivalves besides oysters can be induced by various physical and chemical methods to shed their gametes. Spawning of *Chlamys varia* is initiated by the gametes of the opposite sex and also by a number of mechanical and thermal stimuli such as rough handling, pinching of the mantle, and a sharp increase in temperature. Lubet (1951, 1955) found that thermic stimulation and the extracts of sex cells are more effective in inducing spawning in *Chlamys* than mechanical stimulation and that males are more responsive than females. Similar results were observed in *Mytilus galloprovincialis* and *M. edulis*; but the emission of sperm in these species could not be stimulated by eggs or egg extract, while the fresh sperm or sperm extract are effective in releasing the female spawning. There are indications that spawning of cold water species such as ocean scallop can be initiated by the increase of temperature, but the reaction of this mollusc to other stimuli has not been studied.

Iwata (1952, quoted from André, 1960) was able to provoke spawning in *Mytilus edulis* by electric current and by the injection of a 0.5 M solution of KCl.

Spawning stimuli applied to sexually mature *Crassostrea virginica* are sometimes effective on certain individuals but not on others taken simultaneously from the same population. The lack of response is not associated with insufficient maturity, since all individuals examined in the laboratory were found to have fully developed gonads. A similar condition has been noticed in *Crassostrea gigas* of the Pacific Coast of the United States. Apparently in these cases some unknown inhibition prevented the release of gametes. Lubet (1955) studied this phenomenon in *Chlamys* and *Mytilus* and found that the periods when spawning can be induced by external stimuli coincide with the periods of exhaustion of granules of the neurosecretory cells of the ganglia. The existence of neurosecretory cells in the nerve ganglia of Gastropoda and Scaphopoda was established by the work of Scharrer and Gabe, who also found them in many species of Lamellibranchs (Gabe, 1955). Excision of the cerebral ganglia in *Chlamys* and *Mytilus* provokes precocious spawning and the operated animals spawn much earlier than the controls. Excision of the visceral ganglia seems to retard spawning. Such retardation may be attributed to the isolation of the receptors of the mantle or to the prolongation of inhibitive action of cerebral ganglia. It seems significant that neurosecretion in ganglia cells precedes gametogenesis and that maximum accumulation of neurosecretory products occurs at the time of maturation of sex cells. The demonstrated absence of such secretion in the recently spawned-out animals may be interpreted as a condition which renders them receptive to the spawning-producing stimuli. The release of the inhibition in the molluscs with the excised cerebral ganglia confirms Lubet's hypothesis. His studies indicate that the spawning reaction of bivalves is definitely under the control of the nervous system.

Spawning of many bivalves under natural conditions is usually associated with a rise in temperature, although the exact level of temperature at which different species spawn varies greatly. This led to the assumption of the existence of a critical spawning temperature which acts as a trigger in releasing the germ cells. Observations by several biologists that the spawning of *C. virginica* at different latitudes frequently takes place at different temperatures, usually higher in the south and lower

in the north, was explained by assuming the existence of distinct physiological species or races (Stauber, 1950; Loosanoff et al., 1951). Experimental evidence is not in harmony with this view. It has been shown in my laboratory that ripe adult female oysters taken simultaneously from the upper part of Buzzards Bay respond differently to thermic stimulation. Some of them spawned at a rise in temperature from 19-20° to 22-23°, while others, tested at the same time, required additional stimulation by sperm. Within the same population some individuals were able to spawn at 25-26° C, while others required 30° or even 31-32° C. Oysters unable to spawn at 25-26° C or at higher temperature were induced to spawn at those temperatures by the addition of sperm. It is unreasonable to assume that these individuals of common origin and living together belonged to different physiological species or races. The concept of "critical temperature" is inapplicable to the spawning of the American oyster. The responsiveness of the female to various degrees of stimulation depends on its "critical" state of ripeness rather than on specific temperature. In view of Lubet's findings, it appears reasonable to assume that this state is determined by the presence or absence of neurosecretory products. Only breeding experiments, which so far have not yet been made, can prove the existence of distinct "races" which require different temperatures to initiate ovulation.

Korringa (1957) observed that the three populations of *O. edulis* living at different latitudes release their larvae at three temperature levels (17.5°, 20°, and 25° C) and differ also "in the amount of warmth required for maturation of the sex products." Paradoxically, the population with the highest breeding temperature (25° C) is found at the northernmost fringe of distribution of the species near Bergen, Norway, and the "race" able to breed successfully at the 15° level lives at the southern limit of range near Vigo, in Spain. He explains this strange situation by the hydrographical conditions prevailing in these localities. No experimental evidence was presented by Korringa in support of this conclusion.

A suspension of fresh sperm or eggs always contains a certain amount of blood. It is therefore possible that blood of the spawning female may contain some stimulating substance. In the experiments conducted in my laboratory, the blood was collected from a female in which spawning was induced by raising the temperature. The 1 ml of blood withdrawn from the pericardium was immediately injected into the visceral mass of another ripe, but not spawning, female. The results were negative. Lubet (1955) repeated these experiments using *Chlamys varia* and *Pecten maximus*; he confirmed my finding that a spawning hormone is absent in the blood of the female.

With the greater complexity of the reproductive system of gastropods and cephalopods, their sexual activities become very elaborate. The mating in these molluscs involves several steps beginning with sexual recognition, followed by courting, copulation, deposition of eggs, and parental care of embryos and larvae.

Many marine gastropods have the tendency to climb over vertical surfaces above the bottom. The negative geotropism greatly increases during the breeding season, with the result that eggs of such snails as *Urosalpinx cinerea, Thais haemastoma, Tritonelia japonica,* and others are laid above the bottom on rocks, sticks, sea walls, and various submerged objects. This behavior is successfully utilized by shellfish growers for trapping the snails in wire bags filled with shells and rocks, or by using bamboo sticks placed on the oyster bottom. The latter method is widely employed in the delta of the Mississippi River to control the inroads of *Thais haemastoma,* which is highly destructive to oysters. The laying of eggs by one conch attracts the others and soon several egg-laying females are found on one stick while other sticks nearby contain none. Huge masses of egg cases are collected in this manner.

The highest complexity of sexual activities is reached in land snails and slugs. The

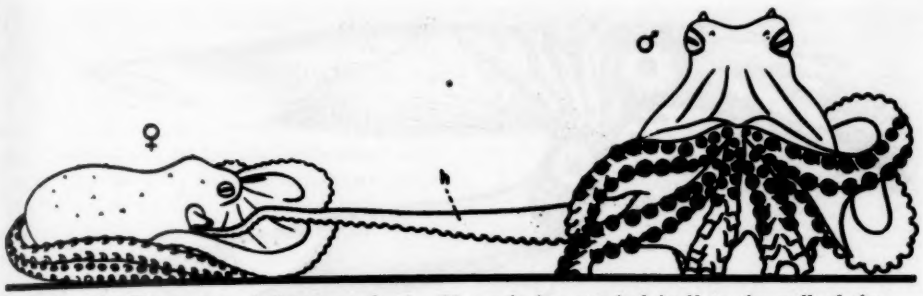

FIG. 11. The mating of *Octopus vulgaris*. The male has attached itself to the wall of the aquarium and extended his hectocotylus (h) into the mantle cavity of the female. From J. Meisenheimer, 1921.

crucial point in the mating of these animals is the transfer of sperm, enclosed in a spermatophore, to the genital opening of the partner. The spermatophore is covered with a chitinous envelope which is secreted inside the tubular outgrowth of the copulatory organ, the so-called flagellum. The pairing animals entwine together for several hours, being enclosed in a copiously secreted mucus. They perform a complicated act which the German zoologists of the past century called "Liebesspiel" or love game. The land snails, *Helix*, at the beginning of the act throw sharp, calcareous arrows, the love darts or "telum amori," and exchange them by lodging these weapons in the integument, sometimes inflicting serious wounds. This behavior precedes copulation and serves to enhance the sexual stimulation. Sometimes the wound is too severe and the partners move away. The actual transfer of spermatophores is assisted by the secretion of special mucous glands near the mouth of the vagina (Meisenheimer, 1907).

The behavior of the hectocotylyzed arm of cephalopods is of great interest, but unfortunately the physiology of this semi-independent organ is not known. One must assume that hectocotyli are equipped with an adequate nervous mechanism for proper orientation in reaching the female and for depositing the spermatophores in the precise position in front of the female's genital opening during copulation.

Courtship and mating of the octopus (Racovitza, 1894b) and *Sepia* (Tinbergen, 1939) starts with the location of the female by the male. As soon as the animals are near each other the male displays optical signals by means of color change, assumes a special posture, and moves its body in a peculiar manner. These actions are apparently recognized by the female (Fig. 11).

The nuptial coloration of *Sepia* is very conspicuous. It consists of zebra-like white and colored stripes on the body and a deep red coloration of part of the head. In assuming nuptial position the male extends sideways the fourth (ventral) arm, changes its color, and widely opens his eye on the side of the outstretched arm. The throwing of large quantities of water, biting and chewing the partner while holding each other in the arms are of common occurrence among the sexually excited animals. Because the color change is due to the contraction and expansion of chromatophores, one may suspect neuro-hormonal control of these reactions; but the problem has not been studied experimentally, probably because of the great difficulty involved in surgical operations on these animals, which are difficult to keep in captivity.

The mating of the common octopus of Europe may last for several hours or even a whole day. Maternal instinct is present. The interesting behavior of a female octopus of the Pacific Coast during mating and in protecting the newly laid cluster of eggs was described by Fisher (1923) and Fox (1938).

The copulation of squids is best known through the work of Drew (1911) on *Loligo*

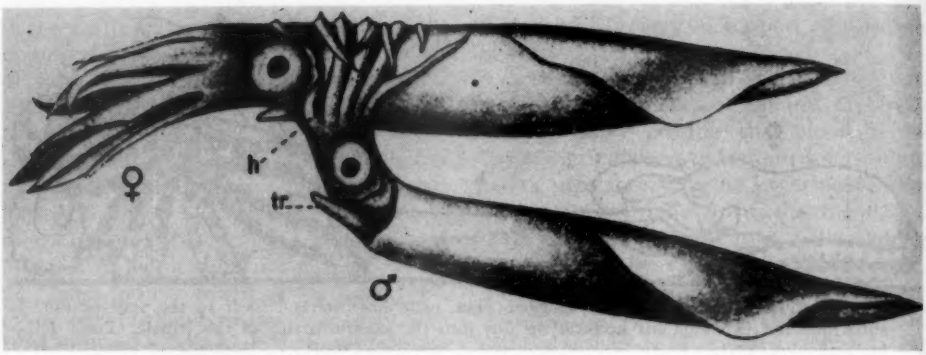


FIG. 12. Mating of squid, *Loligo pealii*. From G. Drew, 1911.

*pealii*. An elaborate courtship precedes mating. During the preliminary act the male squid swims alongside the female, spreading his arm and displaying a dark red color caused by the expansion of its chromatophores. He may come in contact with the female, grasping her around her head with his arms, with the lower sides of both animals in close contact (Fig. 12). At this moment the hectocotylus plucks out a bunch of spermatophores and inserts them into the mantle cavity where they become attached near the oviduct. In another position the two animals join their heads and the spermatophores are placed on the buccal membrane of the female. The squid's spermatophore is a very complex structure equipped with a special reservoir for sperm, ejaculatory apparatus, and a cement body.

The immobile sperm inside the spermatophores are activated by contact with sea water and released through the ruptured reservoir. At this time the eggs are laid and fertilized. Large numbers of spawning males and females, which at other times live separately, gather together and attach large egg masses to the common pile. The fertility of some cephalopods is enormous. Scripps Institute recorded several instances in which fishermen were not able to raise their nets heavily laden with the eggs of the Pacific squid *Loligo opalescens*. On another occasion an area of about 200 acres of sea bottom near LaJolla, California, was covered with the eggs of this species. The European squid, *Loligo vulgaris*, was reported to lay up to 42,000 eggs in one egg mass (Lane, 1960).

### SEX CHANGE

With the exception of the cephalopods, in which the sexes as a rule are separate, the gonads of other classes are unstable and may alternate from male to female or vice versa. Hermaphroditism is encountered in many gastropods and in a few bivalves. The alternations of sexuality in the larviparous oysters and in *Teredo* are probably the best known examples of the sex changes which take place in molluscs.

In the so-called hermaphroditic molluscs (*Ostrea edulis, O. lurida,* and *Teredo*) each normal individual shows a tendency toward rhythmic alternation of functional sexual phases. Several of these phases may be completed in a lifetime, starting with a protandric bisexuality which is generally characteristic for the earliest sexual stage.

Spärck (1925) showed that *O. edulis* in Limfjord, Norway, is a protandric alternating hermaphrodite, in which the sperm-producing period is followed by the period during which eggs are formed, and then again the gonad shifts to the production of sperm. The genital cells not used in the current summer may be utilized in the next breeding period. The interval between the two consecutive female phases is shorter the warmer the water. The sequence of male-female phases is variable (Orton, 1927, 1933) and other combinations such as: male—female—female; male—male—female; and

a rare change of female–male–female take place in *Ostrea edulis* and in *Teredo.*

Histological studies show that the gonad of the American oyster is essentially bisexual. In young *Crassostrea virginica* the cortical layer of gonad follicles consists of ovogonia surrounding the spermatogenic cells. The inherent tendency of oysters toward an alternation of sexuality is probably influenced by environmental conditions aborting one or the other phase. More favorable environment stimulates the growth of ovogonia (Coe, 1936a, 1936b) but neither season, temperature, nor ecological conditions can alter the inherent sequence of sex change. Outside influences may, however, prolong or shorten the durations of sexual phases and affect the number and proportion of the gametes in young oysters (Coe, 1932).

The adult *C. virginica* functions each breeding season either as a male or as a female. Sex change in this species takes place between the two breeding seasons and occurs in a relatively small percentage of the population. In this way *C. virginica* differs from the larviparous *O. edulis* or *O. lurida,* in which the male and female phases follow each other with more or less regular rhythm (Coe, 1934).

The young of *C. virginica* are predominantly male. The percentage of females at the first breeding season is highly variable (from 3 to 33%) in different localities and at different years (Coe, 1936a). An approximate 1:1 sex ratio is usually established in the two-year-old class. In older specimens the sex change is less regular. Needler (1932) and Amemiya (1929) demonstrated this for the adults of *C. virginica* and *C. gigas* respectively, by determining the percentages of sexes in two samples taken during two consecutive years, and by histological examination of excised pieces of gonad and the marking of the individuals which were examined. Injury to the tissues, which might affect the gonad, can be avoided by determining the sex of ripe oysters by inducing each of them to spawn individually and by repeating the procedure in the following years. Using this technique I observed the spawning of 202 adult oysters from Long Island Sound and determined the sex changes that took place in each of these animals during consecutive years. The oysters were four years old at the start of the experiment. Each of them was stimulated to spawn by raising the temperature of the water; the spawning reaction was recorded on kymographs; the sex was determined by microscopic examination of the discharged sex cells; and an identifying number was engraved in the right valve. After the laboratory test was completed, the oysters were returned to the outdoor tanks or placed in cages in the harbor where they were kept until the next spawning season. The procedure was repeated for five consecutive years. The results presented in Table 1 show the changes in the sex ratios which occurred during this period.

TABLE 1. *Changes in the percentage of male and female* Crassostrea virginica *tested consecutively each year*

| Age in years | Males | Females | Not spawning | Total number |
|---|---|---|---|---|
| 4 | 64.4 | 35.6 | 0 | 202 |
| 5 | 65.2 | 32.6 | 2.2 | 181 |
| 6 | 61.2 | 31.6 | 7.2 | 139 |
| 7 | 50.1 | 40.3 | 9.6 | 104 |
| 8 | 44.1 | 41.2 | 14.7 | 68 |

The prevalence of males at the start does not represent the normal sex ratio among four-year-old oysters. The males as a rule are more responsive to stimulation and more readily react to the increase in temperature than the females. Stimulation by adding sperm was avoided in order not to miss the possibility of finding a hermaphrodite. It is apparent that the percentage of females suddenly increased in the seven-year-old oysters. The tendency toward retaining the female phase is more clearly seen by examining the sex-reversed oysters which survived for four years and during this time alternated their sex once or several times. The sequence and frequency of changes in these oysters from male to female or vice versa does not follow a distinct pattern (Table 2).

At the start the group of 31 four-year-old oysters consisted of 23 males and 8 females.

TABLE 2. *Sequence and frequency of sex reversal in the group of oysters which changed sex and survived for four years*

| Sequence | Frequency | Sequence | Frequency |
|---|---|---|---|
| ♂♂♀♀♀ | 4 | ♀♂♀♀♀ | 2 |
| ♂♂♂♀♂ | 4 | ♀♀♀♀♂ | 1 |
| ♂♂♂♀♀ | 3 | ♀♂♂♂♀ | 1 |
| ♂♂♂♂♀ | 3 | ♀♂?♀♀ | 1 |
| ♂♀♂♂♀ | 1 | ♀♀♀♂♂ | 1 |
| ♂♀♂♀♀ | 1 | ♀♀♂♂♂ | 1 |
| ♂♀♀♀♀ | 1 | ♀♂♂♂♂ | 1 |
| ♂♂?♀♀ | 1 | | |
| ♂♂♀?♀ | 1 | | |
| ♂♀♂♂♂ | 1 | | |
| ♂♂♀?♀ | 1 | | |
| ♂♂♀♂♂ | 1 | | |
| ♂♀♂♀♂ | 1 | | |

| | Number of Males | Number of Females |
|---|---|---|
| Start | 23 | 8 |
| End | 11 | 20 |

Question mark indicates failure to spawn.

By the end of the fourth year (the fifth year in which sex was determined) there were 11 males and 20 females. No distinct pattern is apparent in the rhythm of changes except the greater persistence of the female phase. One oyster of the group changed its sex every year: M–F–M–F–M. The inherent tendency toward an alternation of sexuality in the oviparous species is well pronounced among the year-old class (Coe, 1936a) but in a few oysters it persists throughout their life.

The change from male to female is accompanied by changes in the spawning behavior which is manifested in the type of muscular contraction curve and in the discharge of eggs through the gills (Fig. 13). Frequently, however, a fully developed female reaction appears in sex-reversed oysters only toward the middle of the breeding season while the first spawning reaction of the season may still be of the male type, i.e., the eggs are discharged through the cloaca in a male-like fashion (Fig. 14). Toward the end of the breeding season full female characteristics are reestablished and the eggs are dispersed through the cloaca. The change from female to male is always complete with the alteration of sex in the gonad.

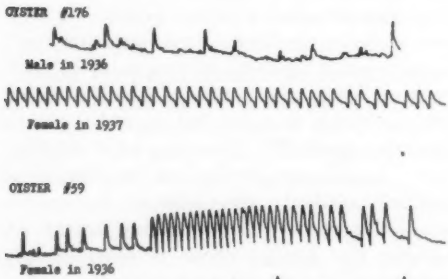


FIG. 13. Spawning reactions of sex-reversed oysters. Complete change in behavior from male to female phase (upper part) and from female to male phase (lower part).

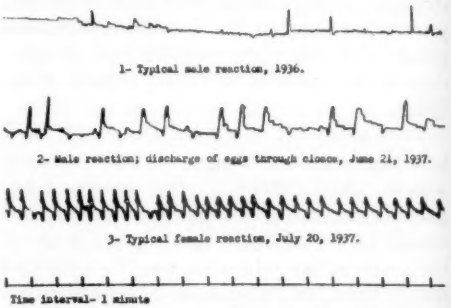
1- Typical male reaction, 1936.
2- Male reaction; discharge of eggs through cloaca, June 21, 1937.
3- Typical female reaction, July 20, 1937.
Time interval- 1 minute

FIG. 14. Spawning reactions of the male oyster which in the following year changed into female but retained male behavior (2nd line). Full female reaction developed later (3rd line).

The spawning record of a hermaphroditic oyster (Fig. 15) combined the characteristics of both sexes. Eggs and sperm were discharged through the cloaca, and the rhythmical contractions of the adductor muscle were not fully developed.

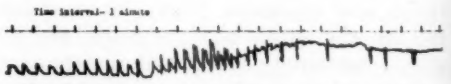


FIG. 15. Spawning of an hermaphroditic oyster.

The development of rhythmic contraction of the adductor muscle of a spawning female and the peculiar position assumed by the pallium are probably due to nervous impulses associated with the release of eggs from the ovary. The spawning syndrome fades toward the end of the breeding season when the major portion of the eggs have

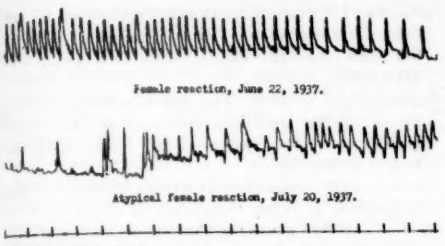


FIG. 16. Change from typical to atypical spawning reaction of a female *Crassostrea virginica* by the end of the breeding season. During the second reaction (lower line) eggs were discharged through the cloaca.

been already discharged. Consequently, nontypical spawning reactions can be observed at the very end of the breeding period. The record reproduced in Fig. 16 illustrates the point. The last and very light spawning of the oyster is atypical; (2nd line), the eggs were discharged through the cloaca and the pallium remained wide open.

The sex change of the gastropod *Crepidula* (Gould, 1952; Coe, 1936c) is of particular interest because of the apparent effect of association of the mollusc with individuals of the opposite sex. *Crepidula fornicata* forms a group of individuals attached in the form of a chain with the youngest and smallest male on the top. The oldest specimens at the bottom of the chain are mature females, while the hermaphrodites are in an intermediate position. Isolation of the male promptly terminates the male phase. The changes from male to female in this and other species can be accelerated or inhibited, but the sequence can not be altered. Coe (1936c) found no evidence of action of a masculinizing hormone which may have emanated from the female. He offered the suggestion (not supported, however, by experimental evidence) that stimuli received by the male through sense organs are of such nature as to influence masculine characteristics, presumably by the mediation of hormones secreted within the body. Sex changes in *Crepidula* offer an excellent opportunity for experimental study of the physiology of sex change, but the problem has not yet been adequately explored.

In several gastropods the change of sex from male to female phase may be correlated with the increase in body length. This has been shown for *Patella coerulea* (Orton, 1927; Bacci, 1947) and for *Fissurella nubecula* and *Calyptraea chinensis,* a species closely related to *Crepidula.* The problem was recently reviewed by Montalenti (1958) and, therefore, will be only briefly mentioned here. In *Patella coerulea* the predominant number of individuals at the beginning of life are functional males. Some of them later change sex and become females, but the transitional phase when both sperm and eggs are formed in the gonad is very short. The correlation between the percentage of individuals in the male phase and body length is clearly shown in the curves published by Bacci (1949). In the case of *Calyptraea chinensis* (Bacci, 1951), the sex change is very abrupt at the size of 6-9 mm when the percentage of males drops to zero. Bacci believes, however, that approximately 8% of individuals of *Patella coerulea* consist of primary females or males. This group comprises small females which did not undergo a previous male phase and large males which apparently are not going to become females. The larger percentage (15%) of individuals that undergo sex change is found in the size class of 20-22 mm. The frequency curve of changes declines symmetrically from this peak to the 7% level in the 14-16 mm class and to about 5% in the 26-28 mm class.

The presence of pure males and females in the population seems to support the hypothesis that sex variability in *Patella* and other gastropods is genetically determined. On the basis of such observations Montalenti and Bacci (Montalenti, 1958) advanced a theory that the time at which sex reversal occurs is mainly determined by a genetic condition upon which environmental factors may exert their role. The effect of environment on sex changes in molluscs is mentioned as a controlling factor by Coe and others, but so far no experiments have been undertaken to determine those factors of environment which influence the frequencies and the direction of sex change.

The physiology of reproduction in molluscs, as may be seen from this review, remains an incomplete and promising chapter of biology.

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# CHEMORECEPTION IN GASTROPOD MOLLUSCS

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"... they had a nice appetite and taste, as well as other creatures, for which purpose they also have gustatory nerves.

"They likewise have a very quick smell: this I observed, when I moved a little fresh food towards them, for they immediately perceived it by the scent, and crept out of their little shells and came to it."

—Jan Swammerdam (1637-1680)
*The Book of Nature,*
English translation, 1758.

## INTRODUCTION

Mechanisms for detecting chemical changes in the environment occur in all groups of animals, and they may be considered in an evolutionary sense as the most primitive distance receptors. Most molluscs, as well as a majority of other animals, are considered to find their food by chemoreception; reliance on this sense in avoiding enemies and finding mates is also considerable. Nevertheless, chemoreception remains the least understood of the higher senses (Ramsay, 1952; Jahn and Wulff, 1950; Nicol, 1960).

There are three approaches to the study of chemoreception: i) anatomical, including the morphology and histology of receptors and their innervation; ii) behavioral, or study of the responses of the animal following subjection of the receptor to chemical stimuli; iii) direct physiological, in which electrical activity in the sensory nerve is studied by amplifying the impulses generated following stimulation and making them visible on an oscilloscope or recording apparatus. Of these the first two have contributed all present knowledge of chemoreception in gastropod molluscs. To the writer's knowledge, the scattered literature on this subject has not been reviewed previously.

In this paper an effort will be made to determine the extent to which present knowledge of the identity, structure, and position of gastropod chemoreceptors may be related to available behavioral evidence and the environmental requirements of the organisms. In addition, electrophysiological experiments which may confirm hypotheses based on the other approaches will be proposed. The aquatic, and especially marine, gastropods will be emphasized in this paper to the virtual exclusion of terrestrial forms. For information on the structure of the chemoreceptors of terrestrial pulmonate gastropods, reference may be made to the recent thorough report of Demal (1955). Studies of behavioral responses of land snails and slugs to chemical stimuli have been reported recently by Kittel (1956), who also reviews the earlier literature.

## DISTINCTION BETWEEN TASTE AND SMELL

Although the distinction between taste or gustation (contact chemoreception) and smell or olfaction (distance chemoreception) breaks down to some extent when aquatic organisms are considered, it remains useful if distance vs. contact, and low vs. high stimulus concentration are used as criteria. There is evidence of distinction between olfactory and gustatory receptors in gastropods, as will be brought out below. When the distinction between olfaction and gustation is based on the physical state of the stimulus (gas or liquid), it is meaningless not only for aquatic organisms, but also at the cellular level in general (Hodgson, 1955).

## CHEMOSENSORY ABILITIES OF GASTROPODS

*Responses to sea water and inorganic ions.* Intertidal gastropods when dry respond positively to sea water splashed on the shell or to immersion. In the limpet *Patella vulgata* and the periwinkle *Nodilittorina granularis,* which have been carefully studied (Arnold, 1957; Ohsawa and Tsukuda, 1955), extension of the head, tentacles, and foot is followed by locomotion if splashing or immersion persists, a response

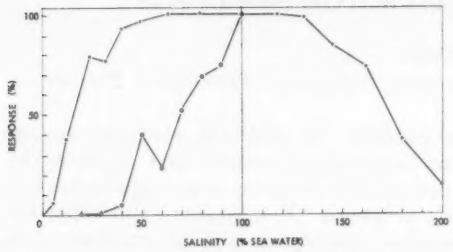



FIG. 1. Positive responses of intertidal gastropods to various salinities. Dots: high intertidal specimens of *Patella vulgata* (Scotland, from Arnold, 1957). Circles: *Nodilittorina granularis* (Japan, from Ohsawa and Tsukuda, 1955).

which is characteristic in nature when splashing from spray increases during incoming tide. In both forms, experimental application of water of varying salinity elicited graded responses (Fig. 1). Ohsawa and Tsukuda obtained parallel linear concentration-response regression lines for *Nodilittorina* when probits of per cent responses were plotted against per cent isotonic concentration (in concentrated sea water) or log concentration (in dilute sea water). The reception concerned is not purely to osmotic pressure, as non-electrolytes in solution failed to elicit the response at any tonicity.

Application of drops of fresh water to the shell of *Patella* elicited the negative response of clamping the shell firmly to the substrate. Exploration of exposed body surfaces demonstrated that application of the stimulus to the cephalic tentacles or mantle fringe was sufficient to elicit the response, while the foot and body wall were insensitive. The interior of the mantle was also insensitive, as was strikingly demonstrated by filling the mantle cavity of an inverted limpet with fresh water; no negative response occurred until it was filled up to the fringe of the mantle. The receptor sites are thus those parts of the body which are in most intimate contact with the environment.

In *Nodilittorina*, the receptor region is on the foot adjacent to the operculum. Application of fresh water to this species did not elicit a response, the individuals remaining as in air with the operculum closed.

In an effort to determine the primary stimulus of the positive response to sea water, Ohsawa and Tsukuda (1955) subjected *Nodilittorina* to isotonic solutions of combinations of salts in the proportions in which they occur in sea water. The positive response was elicited by $NaCl + MgCl_2$, but only slightly by NaCl alone and not by KCl or $CaCl_2$ alone. $MgCl_2$ alone was effective in about half the trials, and $Mg^{++}$ is assumed to be the primary stimulus, although NaCl in the appropriate concentration is also required. $CaCl_2$ caused a negative reaction of withdrawal into the shell, and it inhibited the positive response somewhat when in combination with isotonic Mg-Na chloride solutions. $NH_4Cl$ and KCl were also inhibitory in combination with Mg-Na solution. The response of *Patella* to NaCl solutions of 20-100 g/l (ca. 70-300% sea water) paralleled those to similar concentrations of sea water, although the movements were less smooth and the limpets soon became refractory (Arnold, 1957).

Behavioral responses of gastropods to stimulation with a wide variety of acids, bases, and salts have been recorded, often with little apparent regard for relevance to the habits of the animals, and this knowledge is summarized in Table I.

Except in the case of the nudibranch *Chromodoris*, where direct studies were made, it is difficult to determine the order of stimulating effectiveness of cations, because threshold concentrations were not determined. However, the graded repellent responses of several gastropods to chloride solutions can be roughly analyzed to show the following order for univalent cations:

*Nodilittorina granularis* (Ohsawa & Tsukuda, 1955) $K < NH_4$

*Nassarius mutabilis* (Hoffmann, 1930) $Li < NH_4 < Na < K$

*Physa fontinalis* (Wrede, 1928) $Li < K < Na < NH_4$

*Chromodoria zebra* (Crozier & Arey, 1919) $Na < Li = NH_4 < K$

*Hermissenda crassicornis* (Agersborg, 1922) $Na < NH_4$

TABLE 1. *Behavioral responses of gastropods to inorganic compounds*

| Compound | Concentrations tested g/liter | Concentrations tested Molar | Species | Response* | Source |
|---|---|---|---|---|---|
| *Acids* | | | | | |
| HCl | 1.25– 10 | 0.03 –0.27 | *Nassarius mutabilis* | 2 | Hoffmann (1930) |
| HCl | 0.07– 10 | 0.002 –0.27 | *Physa fontinalis* | 1, 2 | Wrede (1928) |
| HCl | 8.23 | 0.22 | *Hermissenda crassicornis* | 1 | Agersborg (1922) |
| HCN | 10 | 0.37 | *Hermissenda crassicornis* | 1 | Agersborg (1922) |
| $HNO_3$ | 0.75– 30 | 0.012 –0.48 | *Nassarius mutabilis* | 2 | Hoffmann (1930) |
| $HNO_3$ | 0.25– 1.0 | 0.004 –0.016 | *Physa fontinalis* | 1, 2 | Wrede (1928) |
| $H_2SO_4$ | 0.05– 10 | 0.0005–0.10 | *Nassarius mutabilis* | 2 | Hoffmann (1930) |
| $H_2SO_4$ | 0.5 – 5 | 0.005 –0.05 | *Physa fontinalis* | 1 | Wrede (1928) |
| *Bases* | | | | | |
| KOH | 1.0 –100 | 0.018 –1.8 | *Nassarius mutabilis* | 2, 3 | Hoffmann (1930) |
| KOH | 0.5 – 1 | 0.009 –0.018 | *Physa fontinalis* | 1 | Wrede (1928) |
| KOH | 0.17 | 0.003 | *Chromodoris zebra* | 0 | Crozier & Arey (1919) |
| NaOH | 0.5 – 75 | 0.012 –1.88 | *Nassarius mutabilis* | 2, 3 | Hoffmann (1930) |
| NaOH | 0.2 – 1 | 0.005 –0.025 | *Physa fontinalis* | 2, 3 | Wrede (1928) |
| $NH_4OH$ | 8.75 | 0.25 | *Nassarius mutabilis* | 2 | Hoffmann (1930) |
| *Salts* | | | | | |
| $CaCl_2 \cdot 6H_2O$ | 100 | 0.45 | *Nassarius mutabilis* | 1 | Hoffmann (1930) |
| $CaCl_2$ | 1.13 | 0.01 | *Nodilittorina granularis* | 2 | Ohsawa & Tsukuda (1955) |
| $CaCl_2$ | 10 | 1.33 | *Physa fontinalis* | 0 | Wrede (1928) |
| $CaCl_2$ | 69 | 0.625 | *Chromodoris zebra* | 0 | Crozier & Arey (1919) |
| $CuSO_4 \cdot 5H_2O$ | 5 –100 | 0.019 –0.28 | *Nassarius mutabilis* | 2 | Hoffmann (1930) |
| $CuSO_4$ | 10 | 0.05 | *Physa fontinalis* | 2 | Wrede (1928) |
| KBr | 74.5 | 0.625 | *Chromodoris zebra* | 1 | Crozier & Arey (1919) |
| KCl | 10 –100 | 0.135 –1.35 | *Nassarius mutabilis* | 1 | Hoffmann (1930) |
| KCl | 0.72 | 0.01 | *Nodilittorina granularis* | 2 | Ohsawa & Tsukuda (1955) |
| KCl | 10 –100 | 0.135 –1.35 | *Physa fontinalis* | 2, 3 | Wrede (1928) |
| KCl | 0.07– 46.5 | 0.001 –0.625 | *Chromodoris zebra* | 1 | Crozier & Arey (1919) |
| $K_2Cr_2O_7$ | 50 | 0.17 | *Nassarius mutabilis* | 2 | Hoffmann (1930) |
| $K_2Cr_2O_7$ | 20 –100 | 0.068 –0.34 | *Physa fontinalis* | 2, 3 | Wrede (1928) |
| KI | 20 –100 | 0.125 –0.625 | *Nassarius mutabilis* | 3 | Hoffmann (1930) |
| KI | 10 –100 | 0.062 –0.625 | *Physa fontinalis* | 2 | Wrede (1928) |
| KI | 104 | 0.625 | *Chromodoris zebra* | 1 | Crozier & Arey (1919) |
| $KNO_3$ | 10 –100 | 0.099 –0.99 | *Nassarius mutabilis* | 2, 3 | Hoffmann (1930) |
| $KNO_3$ | 63 | 0.625 | *Chromodoris zebra* | 1 | Crozier & Arey (1919) |
| LiCl | 100 | 2.36 | *Nassarius mutabilis* | 1 | Hoffmann (1930) |
| LiCl | 4.7 – 47 | 0.11 –1.11 | *Nodilittorina granularis* | + | Ohsawa & Tsukuda (1955) |
| LiCl | 5 – 10 | 0.12 –0.236 | *Physa fontinalis* | 1 | Wrede (1928) |
| LiCl | 26.4 | 0.625 | *Chromodoris zebra* | 1 | Crozier & Arey (1919) |
| LiCl | 10.6 | 0.25 | *Hermissenda crassicornis* | 0 | Agersborg (1922) |
| LiCl | 10.6 | 0.25 | *Dendronotus giganteus* | 0 | Agersborg (1922) |
| $MgCl_2 \cdot 6H_2O$ | 50 –100 | 0.247 –0.493 | *Nassarius mutabilis* | 1 | Hoffmann (1930) |
| $MgCl_2$ | 4.55 | 0.048 | *Nodilittorina granularis* | 0, + | Ohsawa & Tsukuda (1955) |
| $MgCl_2$ | 59 | 0.625 | *Chromodoris zebra* | 1 | Crozier & Arey (1919) |
| $MgSO_4$ | 10 | 0.083 | *Physa fontinalis* | 0, 1 | Wrede (1928) |
| $MgSO_4$ | 75 | 0.625 | *Chromodoris zebra* | 0 | Crozier & Arey (1919) |
| $MgSO_4 \cdot 7H_2O$ | 246 | 1.00 | *Hermissenda crassicornis* | 0 | Agersborg (1922) |
| NaCl | 20 –100 | 0.19 –1.73 | *Patella vulgata* | + | Arnold (1957) |
| NaCl | 50 –200 | 0.863 –3.45 | *Nassarius mutabilis* | 3 | Hoffmann (1930) |
| NaCl | 27 | 0.46 | *Nodilittorina granularis* | 0, (+) | Ohsawa & Tsukuda (1955) |
| NaCl | 5 –100 | 0.086 –1.73 | *Physa fontinalis* | (2), 3 | Wrede (1928) |
| NaCl | 36.6 | 0.625 | *Chromodoris zebra* | 1 | Crozier & Arey (1919) |
| NaCl | 58.45–116.9 | 1.00 – 2.00 | *Hermissenda crassicornis* | 1, 2 | Agersborg (1922) |
| NaCl | 58.45 | 1.00 | *Dendronotus giganteus* | 1, 2 | Agersborg (1922) |
| $Na_2CO_3$ | 10 –100 | 0.094 –0.94 | *Nassarius mutabilis* | 2, 3 | Hoffmann (1930) |
| $Na_2CO_3$ | 10 | 0.094 | *Physa fontinalis* | 2 | Wrede (1928) |
| $NaHCO_3$ | 10 –100 | 0.119 –1.19 | *Nassarius mutabilis* | 2, 3 | Hoffmann (1930) |
| $NaHCO_3$ | 10 | 0.119 | *Physa fontinalis* | 2, 3 | Wrede (1928) |
| $Na_2Cr_2O_7$ | 2.5 –100 | 0.009 –0.38 | *Physa fontinalis* | 3 | Wrede (1928) |
| $NaNO_3$ | 85 –170 | 1.00 –2.00 | *Nassarius mutabilis* | 2, 3 | Hoffmann (1930) |
| $NaNO_3$ | 85 –170 | 1.00 –2.00 | *Hermissenda crassicornis* | 1, 2 | Agersborg (1922) |

TABLE 1. *Continued*

| Compound | Concentrations tested g/liter | Concentrations tested Molar | Species | Response* | Source |
|---|---|---|---|---|---|
| $Na_2SO_4 \cdot 10H_2O$ | 140 | 0.437 | *Nassarius mutabilis* | 2, 3 | Hoffmann (1930) |
| $Na_2SO_4 \cdot 10H_2O$ | 64.5 | 0.20 | *Hermissenda crassicornis* | 1, 2 | Agersborg (1922) |
| $NH_4Cl$ | 50 –100 | 0.87 –1.75 | *Nassarius mutabilis* | 2 | Hoffmann (1930) |
| $NH_4Cl$ | 2.5 – 20 | 0.044 –0.35 | *Physa fontinalis* | 3 | Wrede (1928) |
| $NH_4Cl$ | 34 | 0.625 | *Chromodoris zebra* | 3? | Crozier & Arey (1919) |
| $NH_4Cl$ | 53.5 –107 | 1.00 –2.00 | *Hermissenda crassicornis* | 0,1,2 | Agersborg (1922) |
| $NH_4Cl$ | 107 | 2.00 | *Dendronotus giganteus* | 1, 2 | Agersborg (1922) |
| $(NH_4)_2Cr_2O_7$ | 5 –100 | 0.020 –0.397 | *Physa fontinalis* | 3 | Wrede (1928) |
| $ZnSO_4 \cdot 7H_2O$ | 10 –100 | 0.035 –0.35 | *Nassarius mutabilis* | 2 | Hoffmann (1928) |

* Responses: 0 No response
1 Mild defensive response: crawling away from stimulus; retraction of parts of body
2 Defensive response: retracts within shell
3 Escape response
+ Positive response: extension of body, locomotion

In a number of other invertebrates and mammals, the order of stimulating effectiveness on taste receptors is $Li < Na < K < NH_4$ (Dethier, 1955, 1956). There are, however, certain prominent exceptions to this generalization (Beidler et al., 1955). Also, attractant and repellent effects of stimuli are likely to differ qualitatively, a factor which reduces the validity of such comparisons with the available information on gastropods.

Ohsawa and Tsukuda (1955) reported an increasing order of stimulating effectiveness of cations $Cl < NO_3 < SO_4$ in *Nodilittorina*, but Crozier and Arey (1919) found that responses of *Chromodoris* to KI, KBr, and $KNO_3$ were comparable to that elicited by KCl and suggested that stimulating capacity is primarily a function of the cation. It was not possible to abstract similar conclusions from the data on *Physa*, *Nassarius*, and *Hermissenda*. The order of effectiveness of anions in uniform cation combination found in other invertebrates and mammals has not been satisfactorily explained (Hodgson, 1955; Dethier, 1956).

*Responses to organic compounds.* Behavioral responses to dissolved organic compounds have been tested in few species of gastropods. The results of experiments which have come to the writer's attention are summarized in Table 2. As in the tests with inorganic compounds (Table 1), there seems to have been little regard for relevance to the habits of the animals. The epitome of such studies may be that of Kempendorff (1942), who demonstrated the attraction of the fresh water pulmonate *Helisoma nigricans* to cooked potato, vanilla pudding, and strawberry jam.

A typical escape response (see below) is induced in *Nassa* (= *Nassarius*) *mutabilis* by chloroform, 0.025 M picric acid, 1.0 M sodium salicylate, and 0.73 M sodium acetate (Hoffmann, 1930). Less violent defensive responses were elicited in the fresh water prosobranch *Viviparus viviparus* by $6.7 \times 10^{-5}$ M quinine (Wölper, 1950) and in several other species by stronger solutions of various compounds (Table 2).

*Nassarius reticulata* responds to dissolved carbohydrates, organic acids, and skatole (Henschel, 1932) by evincing aspects of its behavior pattern typically associated with feeding: movement of the siphon to and fro, movement of the tentacles over the substratum, creeping of the animal toward the stimulus source, and extrusion of the radula. The most effective compound, in terms of threshold concentration, was skatole (beta-methylindole; 0.125 g/l, or ca. $10^{-4}$ M), followed by sucrose, glycine, lactic acid, and maltose (Table 2).

Experiments with subthreshold concentrations showed that mixtures of starch, sucrose, glycine, and glycogen elicited responses, suggesting to Henschel that responses to these substances might be mediated through a single modality or receptor type. In contrast, mixtures of skatole with

starch, glycine, and glycogen did not enhance the stimulating effect, indicating a qualitative difference in stimulus, or a second modality. These results may be roughly comparable to those obtained in studies of insect contact chemoreception, summarized by Dethier (1956), which showed two types of receptors: (i) sugar receptors, sensitive to specific chemical configurations and with a slower response of small amplitude; and (ii) non-sugar receptors, sensitive to a wide variety of compounds including acids, salts, and various organic compounds.

*Chemoreception in mating.* Among aquatic gastropods, the role of chemoreception in mating seems to have been observed first in the functional hermaphrodite *Chromodoris.* A positive response, indicated by successful reciprocal fertilization, was elicited by low concentrations of some secretion but inhibited in higher concentrations obtained in vessels of stagnant water. Removal of the rhinophores did not affect this response. The lips of the proboscis, which is fully protruded and touches the body surface of the partner in courtship, are concluded to be the chief chemosensory regions from observations of behavior (Crozier, 1918; Crozier and Arey, 1919).

Females of several species of slipper limpets of the genus *Crepidula* are known to secrete a diffusible substance which attracts motile larvae or newly metamorphosed juveniles to settle on the shells of the female. In *C. plana,* these settling, sexually undifferentiated young develop into functional males in greater numbers and more rapidly than do young that are isolated or reared in association with large males (Gould, 1919, 1952). The stimulating substance appears to be species-specific, since Coe (1953) demonstrated in choice experiments that sexless juveniles of the two closely related species *C. nivea* and *C. williamsi* were attracted only to adult females of their own species.

Locomotor and general activity in the fresh water prosobranch *Paludina* (= *Viviparus*) *viviparus* is increased by a factor of two when water in which an individual had been present for 3 hours is introduced into a vessel containing a member of the opposite sex. Water from a vessel containing a member of the same sex had no effect. (Table 3; Wölper, 1950.) Chemotaxis may also play a role in gregarious spawning behavior, which has been observed in a number of marine prosobranchs (Hancock, 1959; Kohn, 1961), but this has not been investigated experimentally.

*Chemoreception and homing.* A role of chemoreception in the homing of gastropods has been suggested by Edelstam and Palmer (1950), who attribute homing of marked and transported land snails, *Helix pomatia,* to olfactory cues. When the snails were released at distances of less than 40 m from the point of capture, they returned to the general area of release with an angular error of less than 30°. In contrast, marine limpets, especially of the genera *Acmaea* and *Patella,* may home to an exact spot or "scar" from a much shorter distance, up to 55 cm in *P. vulgata* (Morgan, 1894). In their review, Edelstam and Palmer (1950) conclude that tactile cues are probably more effective guides and chemoreception is discounted in the homing of limpets.

*Responses to predators.* The violent escape responses of species of *Nassarius* when touched by sea stars have been known for many years (Bauer, 1913; Weber, 1924) and were considered to be mediated through chemoreception by Weber (1924) and Hoffman (1930). A similar response occurs when the fresh water pulmonates *Physa fontinalis* and *Lymnaea* sp. are touched by leeches of the genus *Glossosiphonia* (Degner, 1921; Herter, 1929).

When the tube foot or skin of an asteroid or echinoid touches the posterior surface of the foot of *Nassarius reticulata* or *N. mutabilis,* the shell is first catapulted forward so that it lies above the head. Then the anterior portion of the foot is suddenly raised above the substrate and expanded, thus lifting the shell also. Next the foot and much of the body extend out of the shell, which then falls forward and to the right, with its aperture once again directed toward the substrate. This sequence is repeated alternately to left and right up to about 10 times, with the result a rapid escape of the

snail in a zigzag line away from the enemy (Weber, 1924).

Comparable but less dramatic responses have been observed by Bullock (1953) in a wide variety of less agile gastropods, including the limpets *Acmaea cassis, A. limatula,* and *A. scutum*; the keyhole limpets *Fissurella volcano* and *Megathura crenulata*; the abalone *Haliotis cracherodii*; the top shells *Tegula funebralis, T. brunnea, T. ligulata,* and *Norrisia norrisii*; the periwinkles *Littorina planaxis* and *L. scutulata*; and the cowry *Cypraea (Zonaria) spadicea*. Clark (1958) observed similar responses when the top shell *Melagraphia* (= *Monodonta*) *aethiops* was touched by the tube foot of the sea stars *Coscinasterias calamaria* and *Astrostole scabra*.

TABLE 2. *Behavioral responses of gastropods to organic compounds*

| Compound | Concn in sea water used, g/liter | Threshold g/liter | Threshold Molar | Species | Response* | Source |
|---|---|---|---|---|---|---|
| *Sugars* | | | | | | |
| Fructose | 0.5 – 50 | 10 | 0.056 | *Nassarius reticulatus* | + | Henschel (1932) |
| Sucrose | 0.5 – 10 | 1 | 0.003 | *Nassarius reticulatus* | + | Henschel (1932) |
| Sucrose | 342 | — | — | *Dendronotus giganteus* | 1 | Agersborg (1922) |
| Sucrose | 342† | — | — | *Chromodoris zebra* | 0 | Crozier & Arey (1919) |
| Sucrose | 342 | — | — | *Hermissenda crassicornis* | 0 | Agersborg (1922) |
| Maltose | 360† | — | — | *Chromodoris zebra* | 0 | Crozier & Arey (1919) |
| Maltose | — | 10 | 0.028 | *Nassarius reticulatus* | + | Henschel (1932) |
| Lactose | 72 | — | — | *Hermissenda crassicornis* | 0, 1 | Agersborg (1922) |
| Lactose | 180 | — | — | *Dendronotus giganteus* | 1 | Agersborg (1922) |
| *Other carbohydrates* | | | | | | |
| Starch | 0.25– 40 | 0.5 | — | *Nassarius reticulatus* | + | Henschel (1932) |
| Glycogen | 0.03– 2.5 | 0.06 | — | *Nassarius reticulatus* | + | Henschel (1932) |
| *Acids* | | | | | | |
| Formic acid | 5 –100 | — | — | *Nassarius mutabilis* | 2 | Hoffmann (1930) |
| Oxalic acid | 25 –100 | — | — | *Nassarius mutabilis* | 2 | Hoffmann (1930) |
| Oxalic acid | 63 | — | — | *Hermissenda crassicornis* | 1, 2 | Agersborg (1922) |
| Acetic acid | 0.85– 25.5 | 2.2 | 0.035 | *Nassarius reticulatus* | 2 | Henschel (1932) |
| Lactic acid | 0.55– 22 | 1.1 | 0.012 | *Nassarius reticulatus* | + | Henschel (1932) |
| Tartaric acid | 5 –150 | — | — | *Nassarius mutabilis* | 2 | Hoffmann (1930) |
| Tartaric acid | 150 | — | — | *Hermissenda crassicornis* | 2 | Agersborg (1922) |
| Citric acid | 10 –200 | — | — | *Nassarius mutabilis* | 2 | Hoffmann (1930) |
| Citric acid | 96 | — | — | *Hermissenda crassicornis* | 2 | Agersborg (1922) |
| Picric acid | 2.5 – 10 | — | — | *Nassarius mutabilis* | 3 | Hoffmann (1930) |
| Picric acid | 0.02– 1.52 | — | — | *Chromodoris zebra* | 1 | Crozier & Arey (1919) |
| Salicylic acid | 1.8 | — | — | *Hermissenda crassicornis* | 1 | Agersborg (1922) |
| *Other compounds* | | | | | | |
| Skatole | 0.01– 1.0 | 0.01 | $8 \times 10^{-5}$ | *Nassarius reticulatus* | + | Henschel (1932) |
| Glycine | 0.1 – 40 | 0.25 | 0.003 | *Nassarius reticulatus* | + | Henschel (1932) |
| Chloroform | — | — | — | *Nassarius mutabilis* | 3 | Hoffmann (1930) |
| Sodium salicylate | 160 (1.0M) | — | — | *Nassarius mutabilis* | 2, 3 | Hoffmann (1930) |
| Sodium salicylate | 160 (1.0M) | — | — | *Hermissenda crassicornis* | 1, 2 | Agersborg (1922) |
| Sodium acetate · $3H_2O$ | 100 (0.73M) | — | — | *Nassarius mutabilis* | 3 | Hoffmann (1930) |
| Sodium acetate | 82 (1.0M) | — | — | *Hermissenda crassicornis* | 1, 2 | Agersborg (1922) |
| Sodium acetate | 82 (1.0M) | — | — | *Dendronotus giganteus* | 1 | Agersborg (1922) |
| Methyl alcohol | 64 (2.0M) | — | — | *Hermissenda crassicornis* | 1 | Agersborg (1922) |
| Ethyl alcohol | 4.6 (0.1M) | — | — | *Chromodoris zebra* | 1 | Crozier & Arey (1919) |
| Ethyl alcohol | 92.14 (2.0M) | — | — | *Hermissenda crassicornis* | + | Agersborg (1922) |
| Cocaine | 3.03 (0.01M) | — | — | *Chromodoris zebra* | 1 | Crozier & Arey (1919) |
| Nicotine | 4.86 (0.03M) | — | — | *Chromodoris zebra* | 1 | Crozier & Arey (1919) |
| Atropine sulfate | 2.89 (0.01M) | — | — | *Chromodoris zebra* | 1 | Crozier & Arey (1919) |
| Chloretone | 0.89 (0.005M) | — | — | *Chromodoris zebra* | 1 | Crozier & Arey (1919) |
| Curare | — | — | — | *Chromodoris zebra* | 1 | Crozier & Arey (1919) |
| Glycerin | 276 (3.0M) | — | — | *Hermissenda crassicornis* | 0 | Agersborg (1922) |
| Glycerin | 276 (3.0M) | — | — | *Dendronotus giganteus* | 0 | Agersborg (1922) |

TABLE 2. *Continued*

| Compound | Concn in sea water used, g/liter | Threshold g/liter | Threshold Molar | Species | Response* | Source |
|---|---|---|---|---|---|---|
| Quinine sulfate | 0.05– 1.25 | ca. 0.05 | ca. $6.7 \times 10^{-5}$ | *Viviparus viviparus* | 1, 2 | Wölper (1950) |
| Quinine | — | — | — | *Hermissenda crassicornis* | 1 | Agersborg (1922) |
| Orange flavoring | — | — | — | *Hermissenda crassicornis* | 0 | Agersborg (1922) |
| Saffrol | — | — | — | *Hermissenda crassicornis* | 0, 1 | Agersborg (1922) |
| Cedar wood oil | — | — | — | *Hermissenda crassicornis* | 0, 1 | Agersborg (1922) |
| Bergamot oil | — | — | — | *Hermissenda crassicornis* | 0, 1 | Agersborg (1922) |
| Origannum | — | — | — | *Hermissenda crassicornis* | 0 | Agersborg (1922) |
| Clove oil | — | — | — | *Hermissenda crassicornis* | 1 | Agersborg (1922) |
| Vanillin | 0.0001–0.02† | ca. 0.001 | ca. $6.5 \times 10^{-7}$ | *Viviparus viviparus* | + | Wölper (1950) |
| Coumarin | $1.5 \times 10^{-4}$ – $5 \times 10^{-3}$† | $2.5 \times 10^{-4}$ | $1.7 \times 10^{-6}$ | *Viviparus viviparus* | + | Wölper (1950) |

* Responses: 0 No response
1 Mild defense response: crawling away from stimulus; retraction of parts of body
2 Defense response: retracts within shell
3 Escape response
\+ Positive response: extension of body, locomotion

† Concentration in fresh water

Bauer (1913) considered contact with the sea star necessary to elicit the escape response, but Wrede (1928), Hoffmann (1930), and Bullock (1953) stated that it could be induced when the sea star was some distance from the snail. Hoffmann succeeded in inducing the same response by dropping picric acid, chloroform, NaOH, KOH, and salts of Na and K on the posterior region of the foot of *Nassarius mutabilis* (Tables 1, 2). Tube feet of three species of asteroids and *Strongylocentrotus lividus* were effective stimuli. Ophiuroids, crabs, shell and soft parts of the large predatory snail *Dolium*, arms of *Octopus*, and the sea anemone *Adamsia pallasi* were ineffective. Bullock (1953) found that nonpredatory asteroids, such as *Patiria miniata*, did not elicit the response, nor did the echinoid *Strongylocentrotus purpuratus* or the holothurian *Stichopus californicus*. Bullock's observations that the gastropods studied are found in the same area but usually not close to predatory sea stars suggests a possible role of the escape response in determining distribution patterns in nature.

TABLE 3. *Response of Paludina (=Viviparus) viviparus to water inhabited by members of the opposite sex* (From Wölper, 1950)

| Experiment | Sex | Stimulus | Condition of Osphradium | Increase in rate of locomotion* (%) |
|---|---|---|---|---|
| 1 | ♂ | ♀ | intact | 78 |
| 2 | ♀ | ♂ | intact | 72 |
| 3 | ♂ | ♀ | half removed | 72 |
| 4 | ♀ | ♂ | half removed | 61 |
| 5 | ♂ | ♀ | completely removed | 0 |
| 6 | ♀ | ♂ | completely removed | 0 |
| 7 | ♂ | ♂ | intact | 22 |
| 8 | ♀ | ♀ | intact | 16 |

* Numbers represent response expressed as average increase in rate of locomotion of 50 specimens following application of water inhabited for 3 hr by a specimen of the sex indicated in the third column.

A piece of wood or glass rod in contact with the foot of *Nassarius* elicited the escape response only if it had been previously rubbed on a sea star, indicating that mechanical stimuli alone are ineffective. Isolated tube feet were fully effective, but their potency was diminished (Bullock, 1953) or destroyed (Hoffmann, 1930) by boiling in water, suggesting a role of protein.

Violent responses by herbivorous gastropods when touched by carnivorous gastropods have only recently been reported (Clark, 1958). Upon contact with the foot of the New Zealand whelk *Lepsia* (= *Haustrum*) *haustrum, M. aethiops* lifts the shell well above the substratum, swings it violently through an arc of 180°, and crawls rapidly away. In some cases, the much larger predator was actually dislodged. Comparable responses were observed in sev-

eral other species of *Monodonta,* the abalone *Haliotis iris,* and the limpets *Cellana radians* and *C. ornata,* but not in the shield shell *Scutus breviculus* nor the turban shell *Turbo* (*Lunella*) *smaragda.* These exceptions are attributed to the presence of a copious covering of mucus and a thick, calcareous operculum, respectively. Carnivorous gastropods which evoked the response were *H. haustrum, H. scobina, Buccinulum strebeli,* and *Cominella maculosa.* It is not known whether the stimulus is effective at a distance or whether contact is required.

However, introduction of the predatory gastropod *Fasciolaria tulipa* into an aquarium with the herbivorous gastropod *Strombus* evokes a violent escape response, which consists of leaping away from the *Fasciolaria,* in three species, *Strombus gigas, S. costatus* and *S. raninus.* The well known agility of *Strombus* thus seems to be an adaptation to escape from predators (Robertson, 1961). The response is probably to chemical stimuli emanating from the predator, but vision, a well developed sense in *Strombus,* has not been completely ruled out.

The response of certain other freshwater gastropods to leeches is less violent than that of *Physa, Lymnaea* and *Helisoma* mentioned above. The pulmonate *Planorbis* sp. retracts deeply within the shell, a response which is effective if the leech is *Glossosiphonia complanata,* larger specimens of which have thick anterior ends and are unable to reach the retracted soft parts of the snail. However, this defense mechanism is ineffective against *G. heteroclita,* which has a narrow anterior end and can reach and prey on the retracted snail (Herter, 1929). The operculate prosobranch *Bythinella* sp., on the other hand, retracts rapidly, closing the aperature with the operculum, when the soft parts are touched by a leech. The snail remains retracted for some time, during which the leech usually makes several attempts to enter the shell and then abandons the effort (Herter, 1929). Herter's observations suggest that these responses are not mediated by distance chemoreception, as they do not occur when the leech is crawling on the shell. Contact with the soft parts of the snail is required, and it is not known whether the responses differ from those to indifferent tactile stimuli.

An escape response has also been demonstrated in *Helisoma nigricans,* which burrows rapidly in the substrate when specimens of the same species are crushed in the water. Kempendorff (1942) discovered the response when a turtle (*Pseudemys*) introduced into the aquarium ate one of the snails. The others present then dropped off the vegetation or the sides of the aquarium and began burrowing. The response did not occur until the turtle ate a *Helisoma,* suggesting that the effective stimulus is inherent in the snail. It is also species-specific, as crushed individuals of related species do not evoke the response. The response could not be elicited in *Planobarius, Physa,* or *Lymnaea* (Kempendorff, 1942).

*Responses to food at a distance.* A large number of recorded observations of behavior of gastropods in nature suggests that chemoreception is the most important means of food detection among a wide variety of carrion-eating species. Experimental studies of some herbivores and predatory carnivores have led to similar conclusions.

Members of the genus *Nassarius* have long been known to congregate about dead fishes and crustaceans (Cooke, 1895; Dimon, 1905; Copeland, 1918). MacGinitie and MacGinitie (1949) observed *N. fossatus* moving upstream in a current toward a dead fish from a distance of more than 30 m. Another species belonging to the family Nassariidae, *Cyclope neritea,* has been observed to converge on dead crabs and oysters from a distance of several feet (Morton, 1960). The writer has observed *Oliva sayana* moving just beneath the sand substrate to converge on a dead fish from a distance of several meters in a calm lagoon. Cooke (1905) notes anecdotal reports of comparable behavior in *Buccinum, Natica,* and *Neptunea* in nature.

Attempts to obtain more quantitative information have mainly taken the form of preference experiments, in which water is

flowed over the potential food used as stimulus and then over the snails. The chemotactic responses of the latter are measured according to behavioral criteria. In an early experiment, Copeland (1918) found that the mud snail *Nassarius obsoletus* moved upstream in a current which flowed first over lacerated fish (*Fundulus*) 75 cm away. When no fish was present there appeared to be no rheotactic orientation. Feeding responses (cessation of movement and extension of proboscis over substrate) were elicited from *N. obsoletus* in the presence of filtered extract of *Fundulus* muscle (Copeland, 1918).

In the whelk *Buccinum undatum,* which eats mainly "not too old carrion" (Brock, 1953), the siphon is oriented against a current, regardless of the orientation of the animal. If chemical stimuli are placed in the current, the foot turns rapidly in the same direction as the siphon. Brock also showed that *Buccinum* is also able to orient to prey in a completely calm vessel. A piece of fish rubbed on the bottom of the vessel and removed provided the stimuli. A positive response (extension of the siphon and foot and raising of the shell off the substrate) was elicited when the snail was up to 36 cm from the stimulus. Orientation of the siphon, followed by turning of the foot toward a cheesecloth bag containing the soft parts of an oyster, has been recorded in the whelk *Busycon canaliculatum* by Copeland (1918). Copeland was also able to "lead" specimens of *B. canaliculatum* about an aquarium by squirting oyster extract in front of the siphon in the desired direction.

The role of chemoreception in the feeding of herbivorous gastropods has been demonstrated by Van Dongen (1956) in the periwinkle *Littorina obtusata.* The snails were placed in a flow chamber 100 cm downstream from the inflow and stimulus source and the number reaching the source in 12 hours noted. In a large number of trials, significantly greater numbers of *L. obtusata* reached the inflow when pieces of Fucaceae were present than when no algae were present. The differences in attractiveness of different species of *Fucus* and *Ascophyllum* were significant at the .02 level of probability, suggesting that species-specific substances (metabolites?) may be distinguished by the gastropods.

The fresh water pulmonate *Australorbis glabratus* is attracted in a Y-maze to a chemical stimulus diffusing from wheat germ and an alginate-based snail food containing wheat germ (Michelson, 1960). However, the snails were not attracted in the same apparatus to lettuce or watercress, on which they are maintained in the laboratory. *A. glabratus,* which is a vector of *Schistosoma,* feeds on human feces in the laboratory as well as in nature, but evinces no attraction to feces in the Y-maze.

Chemoreception is probably the means by which predatory gastropods become aware of the presence of prey in the vicinity. In *Conus striatus,* a typical feeding response (movements of foot raising the partially buried animal out of the sand; extension of the proboscis) are elicited by introduction into the tank with the snail of water in which fishes, its natural prey, have been swimming (Kohn, 1956). The oyster drill, *Urosalpinx cinerea,* has also been shown to be attracted to sea water which has passed over living oysters (*Crassostrea virginica*) (Haskin, 1950; Blake, 1960).

A number of predatory gastropods appear able to distinguish between chemical stimuli emanating from different potential prey species. Choice chambers, in which water flows over alternate prey organisms and then enters different regions of a vessel containing snails, have been employed to test the responses of several gastropods to different prey species. The nudibranch *Aeolidia papillosa* is attracted to chemical stimuli from the sea anemones *Metridium senile* and *Actinia equina* but not to three other anemones nor to five species of hydroids tested. In contrast, *Cratena aurantia,* another nudibranch, is attracted to the hydroids *Tubularia larynx* and *T. indivisa,* but not to three other hydroids nor five anemones (Stehouwer, 1952; Braams and Geelen, 1953). The prosobranch *Conus sponsalis* is attracted to the polychaete *Perinereis hel-*

*leri* (family Nereidae) more than to *Eunice antennata* or *Palola siciliensis* (family Eunicidae). On the other hand, *Conus abbreviatus* is attracted to *E. antennata* more than to *P. helleri* (Kohn, 1959). In all the experiments cited, the results were correlated with the nature and proportion of the tested prey organisms in the natural diets of the predatory gastropods. These results further suggest that species-specific substances released from potential prey organisms may be distinguished by the snails.

An alternative but not mutually exclusive hypothesis is that gastropods respond to certain external metabolites of their prey in proportion to the concentration of such substances. Blake (1960) has shown that *Urosalpinx cinerea* is attracted in a choice chamber to water which has flowed over prey organisms (the oyster *Crassostrea virginica* and the mussel *Modiolus demissus*) in direct proportion to the rates of oxygen consumption by the prey organisms. These rates could be adjusted in the experiments by varying the number of specimens, by using individuals of different ages, or by using starved or well-fed individuals. When respiratory rates in the two prey chambers were equalized, the preferences of *Urosalpinx* for *Crassostrea* and *Modiolus* were about equal. The results are considered to support the hypothesis that excreted metabolic end products, produced in direct proportion to oxygen consumption, are the chemical attractants to which the gastropods respond. Blake's view is supported by such independent evidence as the preference of *Urosalpinx* for young, rapidly growing oysters, slowly growing oysters transplanted to areas with better growing conditions (Haskin, 1940, 1950), and the positive response of *Urosalpinx* to oxaloacetic acid, an end product in the metabolism of shell formation (Janowitz, cited by Blake, 1960).

Other studies on the food preferences of predatory gastropods have recently been reviewed by Hancock (1960).

*Chemoreception in larval gastropods.* The factors affecting settling and metamorphosis of pelagic larvae of benthic gastropods, and indeed of marine invertebrates in general, are poorly understood, but chemoreception is probably involved. Veliger larvae of the nudibranch *Adalaria proxima* settle and metamorphose only on living colonies of the ectoproct bryozoan *Electra pilosa*. A diffusible substance produced by *E. pilosa* is suggested, as some *A. proxima* larvae will metamorphose on bolting silk which separates them from *E. pilosa* in an aquarium. The larvae do not settle on members of six other genera of bryozoans which were tested unless *E. pilosa* is also present, suggesting that the attractant substance is species-specific (Thompson, 1958).

## SITES OF CHEMORECEPTION

The identification of gastropod chemoreceptors has in the past been based almost exclusively on structural information. Recently, however, several series of experiments in which responses to chemical stimuli before and after extirpation of supposed chemoreceptors have been carried out.

*Rhinophores and oral tentacles of nudibranchs.* On the basis of their position, structure, and innervation, the rhinophores or dorsal tentacles of nudibranchs were concluded to be chemoreceptors by Hancock and Embleton as early as 1849. They reported the confirmatory observation of Alder that the cilia on the rhinophores differed in the direction of beat from those on the branchiae:

> "On these [branchiae] the cilia move constantly from the body towards the extremity of the papilla; on those [rhinophores] they act from the point of the tentacle towards the body; thus in the former case, the water which has served for respiration is drawn from the body and thrown off from the apices of the papillae, whilst in the latter the fluid which we may suppose to contain odorous particles or qualities is attracted to the end of the tentacle, and made to pass down over the entire surface, and thus to act upon the sentient nerve within."

Application of dissolved extract of jellyfish (*Aequorea forskalea*?) to the rhinophores of *Hermissenda crassicornis* elicited a positive response, which consisted of turning of the body toward the stimulus (Agersborg, 1922). The nudibranch bit the pipette if the stimulus was then delivered

toward its head. However, the former response to *Aequorea* extract was indistinguishable from that elicited by touching the rhinophore with a glass rod. Moreover, the feeding response was elicited when the stimulus was placed in front of the head whether or not it was first applied to the rhinophores.

Crozier and Arey (1919) stated that "the rhinophores and oral tentacles are in a general way the most sensitive parts" to chemical stimulation in Chromodoris. Agersborg (1922) found that the rhinophores of *Hermissenda* were more sensitive to acids and salts than the oral tentacles, head, branchial papillae, body surface, and foot. However, he concluded from experiments with food extracts that there was no evidence for distance chemoreception by the rhinophores.

The oral tentacles appear to be the main sites of contact chemoreception in nudibranchs. Those of *Hermissenda* were found to discriminate between food and odorous oils, a feeding response being elicited following stimulation by the former but not the latter (Table 2; Agersborg, 1922, 1925). Agersborg was also able to lead *Hermissenda* about in an aquarium by touching the oral tentacles intermittently with a piece of food. Tests for contact chemoreception by the rhinophores gave only negative results.

*Tentacles, foot, siphon.* In the prosobranchiate and pulmonate gastropods, which do not possess rhinophores, the structures most exposed to the environment are typically the tentacles, siphon, and foot. Piéron (1908) reported the anterior edge of the foot to be the primary site of contact chemoreception with respect to discrimination of food in the pulmonate pond snails *Lymnaea palustris* and *L. stagnalis.* Inert (sand, plaster) or noxious (pepper, quinine) substances, as well as food material, were swallowed indiscriminately when placed on the buccal region. Only nutritive materials which contact the foot are ingested, however; the snail crawls away from other substances (grains of $CaCO_3$, pepper, quinine) applied to the foot. This localization of taste receptors is of adaptive significance, as these species of *Lymnaea* feed on diverse plant and animal matter deposited on the substratum and most likely to come in contact initially with the anterior edge of the foot of the creeping snail.

Hoffmann (1930) showed that the posterior portion of the foot was the site of the contact chemoreceptor in *Nassarius* which, when stimulated by the tube foot of a sea star, causes the characteristic escape response discussed above. Although Bauer (1913) considered the posterior filaments as the receptor site, Hoffmann found that the response was still elicited after their removal in *N. reticulata,* as long as the foot itself was stimulated.

In groups in which the tentacles are rather long but the siphon is short and a long shell siphon is absent, e.g. Nassariidae, the tentacles may be sensitive to dissolved extracts of food material. Copeland (1918) found that filtered extract of *Fundulus* muscle made visible by the addition of powdered carmine elicited a feeding response (cessation of locomotion, extension of proboscis over substrate) when squirted on the tentacle of *Nassarius obsoletus.* Controls, which received sea water and carmine, responded by extension of the proboscis only rarely, and in no case did locomotion cease. However, extirpation of the tentacles of *N. obsoletus,* followed by application of similar stimuli to the anterior edge of the foot, also resulted in a positive response, while controls were positive only rarely. The feeding reaction of the proboscis is thus not dependent on chemical stimulation of the tentacles. In *Viviparus,* removal of the tentacles likewise had no effect on olfactory ability, which is discussed in more detail below (Wölper, 1950). In *Nassarius,* however, it was noted that the positive response occurred only if the siphon clearly entered the colored region indicating the fish extract: "When the siphon was not depressed or swung far enough to reach the extract, the reaction did not take place" (Copeland, 1918). Comparable results were obtained in similar experiments with *Busycon canaliculatum,* which has a long shell siphon and relatively shorter tentacles.

Experiments with *B. canaliculatum*, which were completely buried in sand except for the siphon, showed that stimulation of the siphon alone was sufficient to elicit a feeding response. Removal of the exposed part of the siphon of *N. obsoletus* inhibited the response only when the remaining stub of the siphon was too short to enter the fish extract, whether or not the tentacles were present. Copeland therefore concluded that the response was elicited by stimulation of a receptor located either at the base of the siphon or within the mantle cavity, which all water from the siphon enters, passing over the ctenidium and then past the excretory pore and anus. Copeland was also able to show that *Busycon* is directed toward potential food at a distance on a course indicated by the siphon when it reaches dissolved substances. Although local responses of the tentacles and foot to such stimuli were observed, these structures do not play a role in the orientation of the animal to the stimulus source. His experiments led Copeland to conclude that *Nassarius* and *Busycon* possess a chemoreceptor within the mantle cavity.

*Osphradium.* The osphradium is an organ found within the mantle cavity of all aquatic prosobranchiate gastropods as well as in some opisthobranchs and the basommatophoran (aquatic) pulmonates. It is located generally at the base of the siphon, if one is present, and always directly in the path of water entering the mantle cavity from the environment. In the lower Archaeogastropoda (Zeugobranchia, Patellacea), there is a pair of osphradia, which lie along the anterior portions of the gill supports. Each consists of an elongate ridge of columnar epithelial cells, often with a longitudinal central groove. In the Trochacea and Neritacea among the Archaeogastropoda, and in the Mesogastropoda and Neogastropoda, the osphradium is unpaired and located on the mantle along the base (left side) of the ctenidium. Its form in most Mesogastropoda is similar to that in the Archaeogastropoda. In the Neogastropoda, it is more complex, shorter, broader, and bipectinate (Fig. 2), and placed at the anterior portion of the gill support in such a way that its anterior extremity is directly behind the base of the siphon (Bouvier, 1887; Bernard, 1890; Pelseneer, 1906).

The role of this organ in chemoreception has been debated since it was proposed by Spengel in 1881. Roles suggested by previous authors cited by Spengel included dye secretion, excretion, respiration, and nervous function.

Spengel (1881) initiated comparative anatomical and histological studies on the osphradium, which at that time was usually known as the "supplementary gill," "rudimentary gill," or "false gill." Like Lacaze-Duthiers (1860), Spengel was impressed by the predominantly nervous nature of the organ and concluded that it was not concerned with respiration. Its central tract consists entirely of a thick, elongate ganglion, with central masses of fibers and peripheral cells (Fig. 2). In bipectinate osphradia, 50-200 pairs of leaflets or filaments extend laterally from the central ganglion (Fig. 2). The total surface area may be increased by the leaflets to 15 times the superficial area of the osphradium (Yonge, 1947). Each leaflet receives a prominent nerve from the ganglion, which is overlain by some supporting tissue and a high columnar epithelium continuous with the lower epithelium of the inner surface of the mantle. From its position, innervation, and histology, Spengel (1881) concluded that the main function of the organ was the testing of chemical and physical properties of the respiratory water, including in many cases perception of properties of food.

The term osphradium was applied to "Spengel's olfactory organ" by Lankester (1883), who stated that it "tests the respiratory fluid," an assertion that was subsequently considered authoritative, apparently without further supporting evidence (*e.g.* Bernard, 1890; Piéron, 1909).

In section, each leaflet of the bipectinate osphradium of prosobranchs contains a basal (dorsal) glandular region of mucus-secreting cells, which continue around the lateral margin; a narrow zone of very long,

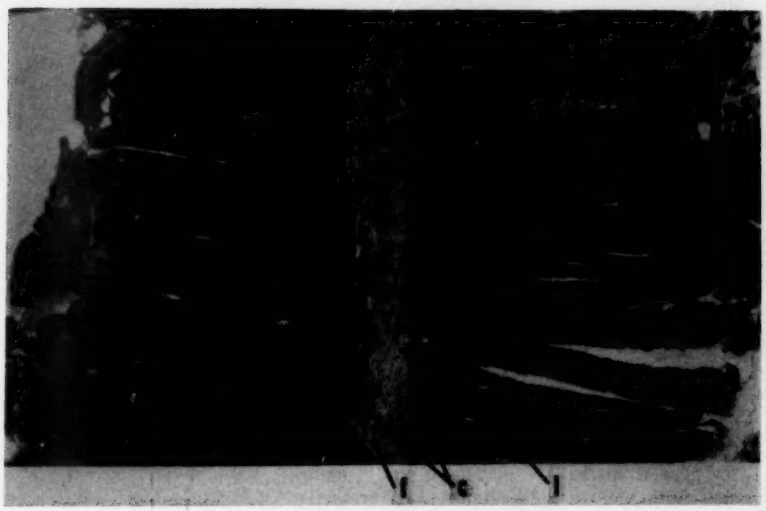



FIG. 2. Longitudinal section of the bipectinate osphradium of *Busycon canaliculatum*. c, region of ganglion cells; f, fibers within central ganglion; l, leaflet or filament. Perenyi fixative, Babes and Patay stains. Preparation by Dr. R. B. Brown.

ciliated columnar cells; and a broad sensory area, occupying the remainder of the surface of the leaflet, the epithelium of which consists of pigmented cells and neuroepithelial cells. In the aspidobranch Archaeogastropoda, these zones are not distinct; and sensory, mucous, and ciliated cells occur mingled in the osphradium (Yonge, 1947). The neuroepithelial cells appear to be found only in the prosobranchs (Bernard, 1890; Yonge, 1947), while the opisthobranchs, like the Pelecypoda, possess only free nerve endings in the epithelium (Stork, 1934). More recently, Demal (1955) has shown the structure of the neuroepithelial cells in greater detail with silver staining techniques. Phylogenetic trends in osphradium morphology are also traced by Demal (1955). Chemoreception by the osphradium of gastropods was first demonstrated by Copeland's successful behavioral studies (1918) following extirpation of this organ from *Busycon canaliculatum* and has been confirmed more recently in two other unrelated prosobranchs (Wölper, 1950; Brown and Noble, 1960) and in one pulmonate (Michelson, 1960).

In a large number of attempted operations on individuals which had been shown previously to respond vigorously to oyster extract squirted over the siphon, Copeland (1918) was able to scrape away the osphradium without damaging the mantle in five cases. Following recovery, the experimental animals, whose behavior was otherwise like that of unoperated controls, were stimulated with oyster juice applied to the siphon tips. In many trials over a period of three weeks, no feeding responses were elicited.

Feeding responses could be elicited from osphradiectomized individuals only when fresh oyster meats were placed in contact with the lower surface of the head. That the stimulus had to be extremely strong is indicated by the fact that it usually did not elicit a response when the head of the snail was under water, or if the oyster was rinsed in sea water before application to a snail out of water. These results show that the osphradium is required for response to dilute food extracts, while very strong stimuli applied to the mouth region also elicit the feeding response. That the latter response is not tactile was demonstrated by positive responses to application of cotton soaked in

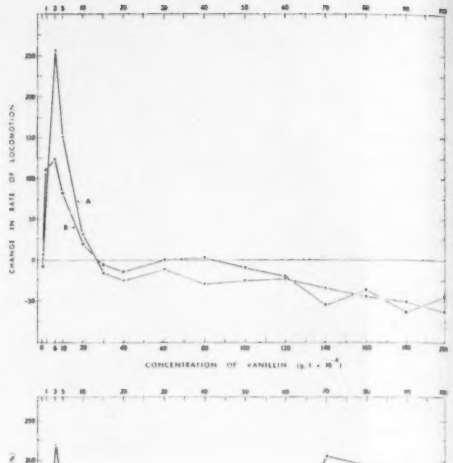
oyster extract but not to cotton soaked in sea water (Copeland, 1918).

Additional evidence in support of the olfactory role of the osphradium resulted from the postoperative survival of Copeland's osphradiectomized specimens for one year:

> "No opportunity of examining them again occurred until a year afterwards, when all six [including one in which osphradium was cauterized] were found alive, in fact indistinguishable in their behavior from other individuals of the species. Upon dropping dilute oyster extract in front of their siphons, they responded to it as they had done before the osphradia were rendered inoperative. Their movements were directed by appropriate stimulations with the extract, they extended their proboscides in the characteristic manner, and again ate oysters which were presented to them on the bottom of the aquarium. The animals were finally anaesthetized and examined, and the fact that their osphradia had regenerated was clearly apparent. Although regeneration of the organs were not complete, several patches of new lamellae covered with pigmented epithelium were present in every instance. Thus with the reappearance of osphradial tissue the scenting reactions returned" (Copeland, 1918).

Although the osphradium is unpaired in the higher gastropods, the typical side to side movement of the inhalent pallial siphon offers the possibility of orientation by comparison of stimulus concentration essentially similar to that of an organism with paired receptors. It is noteworthy that the siphon has developed only in the Mesogastropoda and Neogastropoda, which possess a single, unpaired osphradium. In the Archaeogastropoda (in which chemosensory properties of the osphradium have not been demonstrated) a siphon is lacking and the osphradium is paired in the more primitive groups.

To the writer's knowledge, no attempts were made to confirm or extend Copeland's results until the experiments of Wölper (1950) on *Viviparus viviparus,* from which osphradia were partially or completely removed by cauterization. Although food is not sought in this species by goal-directed movements, the rate of locomotion of intact animals increases in the presence of food or a chemically attractant substance, such as vanillin or coumarin (Table 2). Active



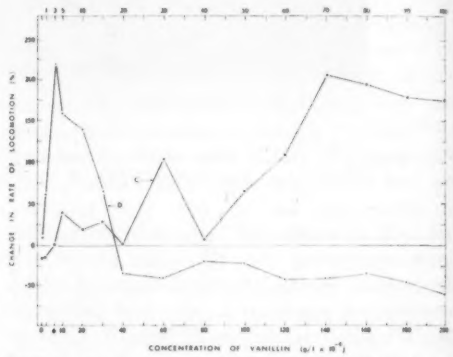




FIG. 3. Responses of *Paludina* (= *Viviparus*) *viviparus* to vanillin. The results are expressed as change in rate of locomotion following application of the stimulus. A (dots), osphradium intact; B (circles), half of osphradium removed; C (circles), osphradium completely removed; D (dots), osphradium regenerated (4 weeks after complete removal). Each point represents the average of ten trials with different specimens. The upper abscissas show the concentration in per cent of saturated vanillin solution added to 500 ml of water which contained each snail. From Wölper (1950).

searching movements of the buccal complex also occur. There is an optimum concentration of chemical attractant, *ca.* $6 \times 10^{-4}$ g/l in vanillin, above and below which the response diminishes (Fig. 3, curve A). When half of the osphradium was removed, the response was similar to that of intact animals (Fig. 3, curve B) although the amplitude was lower; these animals served as surgical controls.

Fully osphradiectomized individuals responded slightly to concentrations of $2 \times$

$10^{-3}$-$8 \times 10^{-3}$ g/l and more markedly to an optimum concentration of $1.4 \times 10^{-2}$ g/l (Fig. 3, curve C). In these cases the responses are considered to follow stimulation of other chemosensory cells whose sensitivity is less than that of the osphradial cells. Following regeneration of the osphradium, which required only four weeks, the responses (Fig. 3, curve D) were essentially similar to those of intact animals (curve A).

Wölper (1950) also demonstrated that the response of *Viviparus* to secretions of the opposite sex, mentioned above, is mediated by the osphradium. As shown in Table 3, the response was completely inhibited by total osphradiectomy.

Thus the function of the osphradium as a distance chemoreceptor, first shown by Copeland (1918) in the neogastropodan *Busycon*, has also been demonstrated in the order Mesogastropoda. Additional confirmation of this function in the Neogastropoda has been provided by recent experiments on *Bullia laevissima* (Family Nassariidae). In 124 attempts, Brown and Noble (1960) successfully severed the osphradial nerve without damaging other structures in 13 specimens. In contrast to controls, these individuals remained partially buried in sand when presented with preferred food, the tunicate *Pyura*. They did feed, however, when touched by the food, indicating that severing of the osphradial nerve affected only distance chemoreception.

Recently, Michelson (1960) has demonstrated chemoreception by the extrapallial osphradium of the fresh-water pulmonate *Australorbis glabratus* in a manner similar to Copeland's experiments. In a Y-maze, a highly attractive alginate-based snail food (see above) was selected by normal and sham-operated controls, while osphradiectomized (by cauterization) individuals behaved randomly. Random responses also resulted following destruction not of the osphradium itself but of its ganglion, which in contrast to the case in prosobranchs is a discrete structure lying below the osphradium.

The role of the osphradium in chemoreception has been questioned by Hulbert and Yonge (1937; Yonge, 1942, 1947), mainly because of an absence of correlation between presence and size of osphradium and need for "water testing." The organ is well developed in some browsing herbivores, deposit feeders, and plankton feeders among the Prosobranchia. These authors proposed that the osphradium functions as a tactile receptor which estimates the amount of silt entering the mantle cavity in the respiratory current. Yonge (1942) found that particulate matter carried in the inhalent current does impinge on the osphradium in the heteropod *Oxygyrus keraudreni*. Yonge (1947) stated that there is some correlation between size of osphradium and degree of sedimentation to which the animal is exposed, and cited the prominent osphradia of *Buccinum, Fusus, Nassa* (= *Nassarius*?), *Murex*, and *Conus*. However, there also appears to be a correlation between size and predatory feeding habits. Species of *Conus*, for example, do not live in silty places but they are carnivorous (Kohn, 1959). At the present time, Yonge (*in litt.*, 1960) is of the opinion that the silt-detecting function of the osphradium is probably primitive but that the organ may have acquired the second function of chemoreception in the carnivorous Mesogastropoda and Neogastropoda.

The writer in collaboration with Dr. Hideki Tateda attempted to record action potentials from the osphradial nerve of *Busycon contrarium* and the horse conch *Pleuroploca gigantea* while various test solutions (oyster extract, starch, amino acids) were flowed over the osphradium. We observed spikes on the oscilloscope occasionally but obtained no definitive data. Difficulties are imposed by the likelihood of injury in removing sufficient shell to expose the osphradium and nerve, and by the presence of a layer of mucus over the surface of the postulated receptor. The absence of such mucus is an important advantage in the study of arthropod chemoreceptors, but its presence in mammals has not been an insurmountable obstacle (Hodgson, 1955; Beidler, 1958, 1960). The failure to obtain

reproducible records of electrical activity in the osphradial nerve is not considered as evidence against a chemosensory function of the osphradium in the species studied.

## SUMMARY AND CONCLUSIONS

Chemoreception in gastropod molluscs seems to have been first discovered by the great Dutch naturalist Swammerdam in the 17th century. Despite the considerable interval since then, the probability that chemoreception is paramount in providing gastropods with sensory information, and the structural simplicity of chemoreceptors in comparison to other sense organs, little is understood about the receptors or the specific, effective stimuli involved. The evidence presently available has been obtained from behavioral studies. These have demonstrated that some herbivorous, carrion-feeding, and predatory gastropods are attracted by chemical stimuli emanating from food. Some of the more agile gastropods respond to chemical stimuli from enemies by fleeing. Both attractant and repellent responses to dissolved inorganic and organic compounds have been reported and are summarized in Tables 1 and 2. The attraction to organic compounds such as carbohydrates and amino acids may be related to chemical attractants from food. More and better controlled ethological studies would provide considerably more information on gastropod chemoreception.

Structural resemblances of rhinophores and osphradia to known chemoreceptors of other animals include neuroepithelial cells or free nerve endings and extensive innervation. In addition, these structures resemble mammalian olfactory organs in that much of the receptor surface is exposed not to the main fluid current but to secondary or "eddy" currents (Jahn and Wulff, 1950). Chemosensory functions of the osphradium predicted on morphological grounds have been confirmed by observations of behavior before and after extirpation of the organ in four species of gastropods, three prosobranchs and one pulmonate.

It is hoped that others will be encouraged to investigate the responses of gastropod chemoreceptors by electrophysiological methods. It has been only within the past decade that action potentials generated by invertebrate chemoreceptors have been recorded, and all studies to date have been concerned with arthropods (Barber, 1951, 1953, 1956; Hodgson and Roeder, 1955; Hodgson, 1958; Wolbarsht and Dethier, 1958; Case, et al., 1960). Behavioral results indicate that some gastropod chemoreceptors occur within the mantle cavity in the current of respiratory water, while others are present in those parts of the body which are in intimate, direct contact with the environment, such as tentacles, mantle fringe, and foot. It is suggested that electrophysiological techniques will provide the clearest demonstration of the chemosensory role of these organs.

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